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SESSION 1 : Applied Technologies

Application of chitosan and essential oils as alternatives fungicides for suppression post harvest fungi of citrus fruits

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Chitosan and essential oils such Lemongrass and citral individually or in combination were tested against *Penicillium digitatum* and *P. italicum* the main pathogens of rotting citrus fruit. *In vitro* , concentrations of chitosan ,lemongrass, citral and chitosan-oil mixtures significantly reduced the linear growth and spore germination of *P. digitatum* and *P .italicum*. Complete growth inhibition for *P.digitatum* and *P.italicum* was obtained with chitosan at 6 g/L, lemongrass oil and citral at 6 ml / L , chitosan +citral or lemongrass oil mixtures at 3g/L+ 3 ml / L or 4 g/L+ 4 mL / L. *In vivo* trials, Coated orange and lime fruits with such previously treatments caused significantly protection of orange and lime fruits green and blue moulds diseases during 60 days of storage. Combination between chitosan and essential oils as fruit coating could be applicable safely for controlling post harvest decay of citrus fruits.

Key words: Essential oil – Chitosan -Post harvest - Citrus fruits.

Potential use of nanocomposites with essential oils for active packaging

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Nanotechnology and essential oils have enhanced the production of active packaging. This study looked into the bacteriostatic properties of different essential oils in their nanocomposites against common food microorganisms, such as *Bacilius cereus, E. coli, Staphylococcus aureus* and *Pseudomonas aerogenosa* . The antibacterial properties were established using disk assay technique and sensory evaluation was conducted to determine the influence of the nanocomposite with essential oils to color, aroma, texture, general appearance

and acceptability. Results of the study showed that among the essential oils studied, red thyme greatly inhibited the growth of all test organisms; while the others inhibited growth of some bacteria except *P. aerogenosa* with eucalyptus; *E. coli* with lemon and rosemary; and *S. aureus* with peppermint . Furthermore, polylactic acid nanocomposite with different essential oils also showed antibacterial properties against the common food borne organisms suggesting the promising potential for active food packaging utilization. Preliminary sensory evaluation further showed varying influences of the nanocomposites with essential oils on the color, aroma, texture and general appearance and acceptability of the meat .

Keyword: active packaging, nanocomposite, essential oils, antimicrobial activities, polylactic acid

Effect of benzyladenine (BA) on *in-vitro* multiplication of *Musa* (AAA group) 'Kluai Nak'

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In vitro Multiplication of *Musa* (AAA group) 'Kluai Nak' was conducted in Murashige and Skoog ((1962medium supplemented with -6Benzyladenine (BA) of concentration as 3 1 0 and 5 ppm. Planned trial Completely Randomized Design (CRD). Subculture was undertaken every 4 week is duration 12 week. It was found that the hight of explants was 2.83 cm. respectively on MS medium with 0 ppm BA, that statistical highly difference significantly on the other media. MS medium with 1 ppm BA has the number of shoots was 2.42 shoots that statistical highly difference significantly on the MS medium with 0 and 5 ppm BA while MS medium with 3 ppm BA has the width and length of explants were 1.39 and 1.49 cm respectively, that statistical highly difference significantly on the MS medium with 0 ppm BA. The MS medium supplemented with 1 ppm BA was to investigate for multiplication of Musa (AAA group) 'Kluai Nak'

Key words: Banana , *Musa* (AAA group) 'Kluai Nak', Benzyladenine, *In vitro* Multiplication

Study on the use of rectangular and triangular models for hydroponics in the culture of *Anubias barteri* var "Broad Leaf"

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Hydroponics is a method of culturing plants without the use of soil. *Anubias barteri* var "Broad Leaf". is an aquatic plant being cultured as a decorative plant in aquaria. It is in demand and is expensive. This research was conducted to compare the use of rectangular and triangular models for the culture of *Anubias barteri* var "Broad Leaf". Specifically, to compare the leaf width, leaf length, leaf thickness, number of leaves per plant, chlorophyll content and fresh weight at 120 days after planting. Results revealed that no significant difference was observed among plants in terms of all the parameters studied within each model except for number of leaves per plant in the triangular model. In comparing the two models, no significant difference was observed in all parameters except for plant height wherein plants grown in triangular model were taller than plants grown in the rectangular model.

Key words: hydroponics, Anubias, model

A review on quality function deployment for agro-product

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Quality function deployment (QFD) is the technique widely used for product and service development. This technique begins by collecting the voice of customers and converts customer requirements into technical specifications or features that need improvement. The ideas come from the involvement of employees in the organization. QFD technique is not limited to use in the design and development of products or services. It can also be used in quality management, analysis of customer needs, management decision-making, planning, timing and cost reduction, etc. The survey, in 2002, found that QFD technique was applied and presented in 650 academic papers worldwide. In Thailand, QFD technique is used for development of products,

curriculum, training courses, residence construction planning, improving the quality of service in library and hospital. For agricultural product, QFD is used in many ways. Since agricultural products are sensitive to the needs of the consumer. This paper presents a detail literature review on the topic of using QFD on agricultural product. The descriptive of methodologies, the benefits, the challenges of applying QFD are discussed and presented.

Key words: quality function deployment, consumer

Participatory democracy of Phetchaburi community organization council

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This study investigated the participatory democracy of Phetchaburi community organization council. Specifically, it aimed to study on participatory democracy of community organization council and the understanding of participatory democracy of community organization council. The study utilized the interview schedule with open-ended questionnaire on participatory democracy and understanding of participatory democracy was used as data-collected method. A sample of 34 cases was drawn from 148 members of community organization council carrying out over 37 Tambon using simple random sampling method. The findings revealed that understanding of overall members toward participatory democracy in term of definition was insufficient while there were 6 percentages of all members mentioning extension of people rights and freedom protection in term of such system attributes seen as small parts of the system. In term of deliberative democracy characteristics, the finding found that lack of profound understanding on participatory democracy led to the problem on adhering to personal satisfaction and personal sake, overlooking others' opinion, lack of responsibility, lack of enough proposed data, lower community consciousness, lack of self-confidence, and resistance to changes. Based on the findings, the intention of drafting community organization council for fundamental democracy system development seems to be out of reach due to practical failure resulting lack of profound understanding what the bill legislated.

Key words: Participatory Democracy, Community Organization Council, Deliberative Democracy

In-vitro testing of nanomaterials containing *Chaetomium globosum* ethyl acetate extract against *Fusarium oxysporum* f. sp. *lycopersici* (race 2)

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The use of nanomaterials in agriculture is just new. This research was done to find an alternative method of controlling Fusarium wilt in tomatoes caused by *Fusarium oxysporum* f. sp. *lycopersici* (race 2) using different concentrations of nanomaterial containing ethyl acetate crude extract from *Chaetomium globosum*. Specifically it aimed to determine and compare the colony sizes among treatments as well as the number of spores. Results show that the effect of 10 ppm nanomaterial containing ethyl acetate crude extract is not significantly different from the use of 1 ppm pure in terms of colony diameter at 7 days of incubation. The percentage reduction of spores was found to be 88.89% in 5 ppm nanomaterial, 90.66% in 10 ppm and 94.68% using pure compound. The ED50 was calculated to be 1.37 ppm using crude extract while the ED50 using nanomaterial was found to be 0.0093 ppm. The occurrence of constricted ad empty spores was also observed.

Key words: nanomaterial, Fusarium wilt, tomatoes

Tropical fruit wine processing: An approach to nurturing the entrepreneurial culture among students

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This project sought the significance of several less valued tropical fruits that can be processed into wine such as makopa, mabolo, duhat, banana, tomato and bugnay. Wine processing in Quirino State University was established by the Quirino Young Entrepreneur's Association (QYEA) a recognized student's organization spearheaded and managed by the agriculture students which main goal is to serve as an avenue to financially challenged students earns additional allowance while enhancing their entrepreneurial and marketing skills. The products' name is JAVEZ which was derived from the name of the first manufacturers. The wines are packaged in bottles with attractive and informative labels after going through the initial stages

of preparation of yeast culture up to the fruit wine processing. Initial produce was very limited in volume due to the very limited source of capital which is out of the students' money. During the group's various displays and product exhibits in provincial and regional events, the Department of Labor and Employment sought the feasibility of the product thus providing the group with additional fund amounting to Php 216,000.00 for the enhancement of the entrepreneurship laboratory, provision of the processing equipment, packaging materials, labels and other materials thereby making the enterprise self-sufficient. Moreover, the laboratory is utilized as a training ground of students taking up entrepreneurship and marketing courses to further develop their skills in wine processing and related activities. The group was able to acquire a Return on Investment of more than 50% from the estimated cost depending on the kind of fruit used. Continuous monitoring and evaluation is done by the project management and adviser. A financial journal and income statement is submitted to the auditor and advisers to assure sustainability of the project through profit sharing. All sales are deposited in the bank in the name of the association care of the president and the treasurer wherein 70% is divided among members, 20% goes to savings and 10% for maintenance. The QYEA continuously operates as a successful business enterprise developing future entrepreneurs who creates employment contributory to economic development .Wine products produced has been acknowledged by the Department of Science and Technology and the primary producer of local wines in Quirino and nearby provinces. Marketing strategy employed includes the integration of wine's health benefits through word-of-mouth, fliers, bulletin advertisements and holiday promotions.

Key words: fruit, wine, processing

Analgesic and antipyretic potential of an ethnobotanical plant, *Alstonia scholaris* L. in mice

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Medicinal plants have been used for treatment of diseases since ancient times In addition, plants are cost effective and less harmful than the synthetically produced drugs. Therefore, there is a need to evaluate the medicinal properties of plants. The study aims to determine the analgesic and anti- pyretic property of the A. scholaris leaves from Brgy. Sta Fe, Imugan , Nueva Vizcaya, Philippines. A. scholaris leaves were extracted by hot water extraction (HWE) technique. A setup of thirty mice was used for the two tests with three treatments each with five replicates. Based on the results of the study, A. scholaris HWE is significantly comparable to the positive control. Hence, A. scholaris HWE has analgesic and antipyretic properties.

Key words : antipyretic, analgesic, ethnobotany

Bag opening technique for bag spawn culture of spit gill mushroom (*Schizophyllum commune*)

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Schizophyllum commune (spit gill mushroom), the wood decay is the most beneficial mushroom. It has been known as the medicinal mushroom. It contains high essential medicinal compound, anti-oxidant, anti-cancer polysaccharide and producing anti-bacterial secondary metabolites. Bag opening technique was conducing to enhance produce of bag spawn mushroom culture by using sawdust as culture medium. Vertical cutting, 45 degree skew cutting and cutting stripe number were compared for fruiting body productivity. The result reveals that 5 stripe 45 degree skew cutting per bag enhanced the highest produce (fresh weigh) of 187.76 g/ bag. However, it was not significantly different from 4, 5 and 6 stripe vertical cutting of 180.39, 179.39, and 176.67 g/bag respectively but, it was significantly higher than 5 stripe 45 degree skew and 6 stripe vertical cutting of 157.20 and 54.11 g/bag respectively. This technique enhanced mushroom productivity per dry weigh of spawn was 31.29 %. The cost and return per bag were 11.26 and 16.90 baht; and benefit was 8.45 baht/bag with the return on investment 75.09 %.

Key words: spit gill mushroom, *Schizophyllum commune*, bag opening technique, bag spawn culture

Effects of gibberellic acid on fruit growth and fruit development of mangosteen (*Garcinia mangostana* Linn.)

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The different concentrations of gibberelic acid (GA_3) applied by spraying on the 15-year-old magosteen trees during the full bloom and fruit setting stage. The study was conducted at the Chumphon Research and Training Centre, Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Chumphon province from Mach, 2009 to Mach, 2010. The experiment

was used the Completely Randomized Design (CRD) with 5 replications. The different GA_3 concentrations were evaluated: 0 25 50 75 and 100 ppm. The results of the study showed no significant differences among treatment means between GA_3 applied in different concentrations with control in parameter of fruit size (width and length) and fruit weight during fruit growth and fruit development at 15-45 days. After 60-120 days, the result showed differences among treatment means between GA_3 applied in different concentrations with control in parameter of fruit size (width and length) and fruit weight. Also, the different concentrations of GA_3 was affect of peel weight and aril weight . The total soluble solid (TSS) was recorded data that there is no significance found on the sweetness of fruit obtained from both treated and untreated trees.

Key words : Mangosteen, Gibberellic acid, GA_3**,** Fruit growth**,** Fruit development

Development of instant chevon "papaitan"

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The study was conducted to produce highly acceptable instant "papaitan" from chevon. Optimum drying condition and standard formulation for the production of instant chevon papaitan were established. Drying rate, as affected by type and level of humectants used, was evaluated. Physico-chemical and microbiological properties of the product were determined to know the effects of the treatments on stability and acceptability. Drying runs showed that cooked chevon papaitan soaked in humectants specifically 1% glycerol, 1% propylene glycol, 2% glycerol and 2% propylene glycol dry more rapidly than the control (without humectant). Treatment with 1% propylene glycol has the fastest drying rate. There is no difference between treatments in terms of bulk density, rehydration characteristics and water activity. Instant chevon "papaitan" samples have water activity of less than 0.3 indicating stability. Aerobic plate count (APC) of the product for yeasts and mold which is <10 estimated APC also confirmed that the product is microbiologically safe and stable.

Key words: instant chevon "papaitan", humectants, drying rate, water activity, bulk density, rehydration characteristics

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Influence of chocolate texture on Belgian consumer's emotions and affective ratings

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This study aimed to determine the influence of texture on Belgian consumer emotions and their affective or hedonic ratings. This was explored using commercial brands of chocolates by linking instrumental analyses: colorimetry, texture analysis and differential scanning calorimetry (DSC), descriptive sensory analysis by QDA® with a trained panel (n=8), consumer acceptance testing and emotion testing by the EsSense Profile TM methodology using a consumer panel (n=126). Results revealed significant differences of the chocolate samples in terms of instrumental and sensory characteristics especially in terms of textural characteristics. Cote d'OrTM was found to be the hardest both in the instrumental hardness test and QDA® while Koetjesreep is the softest. QDA® also showed that Cote d'OrTM is most intense in brown color, cocoa aroma, snap, hardness, bitterness, creaminess and oily film formation among the three samples which is the opposite of Koetjesreep and had intense grittiness and amount of residuals. Consumer testing showed that Cote d'Or was the most acceptable sample. The EsSence Profile TM revealed that each type of chocolate had a distinct emotional profile. Correspondence analysis showed that textural characteristics such as hardness, snap, oily-film formation were positive drivers of liking while grittiness and residuals influenced overall acceptability in a negative way. More insight was provided by the emotions where Cote d'Or was associated with the most number of positive emotions such as 'eager', 'energetic', 'enthusiastic', 'free', 'glad', 'happy', 'joyful', 'pleased', 'satisfied', and 'whole', most of which were significantly different from the other samples. KoetjesreepTM on the other hand was associated with most of the negative emotions (bored', 'disgusted' and worried) but was the only chocolate associated with the 'nostalgic' emotion since it is the oldest brand of chocolate from the group which respondents associate with their childhood. The overall acceptability and emotional profiles of chocolates in this study are influenced by textural characteristics which can be used for competitive marketing in the chocolate industry.

Key words: chocolate, sensory, emotion

SESSION 2: Taxonomy and Diversity

Isolation and morphological study on microorganisms from goat's stomach

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A study on the isolation and morphological of microorganism from the goat's stomach was conducted. The microorganism were collected from 4 parts of stomach as rumen, reticulum, omasum and obomasum. The objective to identification and study on morphorlogical of microorganism, which have isolated in stomach of goat. The isolation and identification on microorganism from stomach of goat that have 2 method for isolated fungi by soil plate techniques and soil dilution plate technique. Results shown that bacteria gram positive were G-RET-01-1, G-OMA-01-1 and G-ABO-02-2. The bacteria gram negative were G-RU-01-1 and G-ABO-01-1. For the Actenomycetes gram positive were G-RET-01-1, G-OMA-01-1 and G-ABO-01-2. Actenomycetes gram negative were G-RU-02-01, G- RET-02-1 and G-ABO-01-1. For the fungi which have isolated by soil plate techniques were found *Aspergillus niger* G-RU-01-1, *Aspergillus flavus* G-RU-01-3, *Aspergillus fumigatus* G-RU-02-2, *Trichoderma* sp. G-RU-02-1, *Mucorales* sp. G-OMA-01-1 and *Penicillium* sp. G-OMA-02-1. The fungi which have isolated by soil dilution techniques were found *Aspergillus niger* G-RU-02-1, *Aspergillus fumigatus* G-RU-02-3, *Aspergillus flavus* G-RET-02-1 and *Mucorales* sp. G-OMA-02-1.

Key words: ruminant, rumen, stomach microorganisms

Evidence of molecular marker for genetic relationship of *Asystasia gangetica* (Linn) T. Anderson

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Asystasia gangetica (L.) T. Anderson (Acanthaceae) is widespread throughout the old world tropics. The plant is cultivated as an ornamental and various parts of the plant are also used traditionally for many ailments and diseases. Two subspecies are recognized in Thailand (1) A. gangetica micrantha that flowers are mostly white with two parallel purple blotches lines on the bottom petal lobe and (2) A. gangetica gangetica that flowers are various shades color as a pink, mauve or purplish with paler throats and white or cream with pale yellowish throats. However, there are comparatively a lesser number of reports focusing on intraspecific genetic diversity studies. The genetic diversity of a local subpopulation of A. gangetica was surveyed using random amplified polymorphic DNA (RAPD) analysis. A total of sixty-four random primers were used and eight primers (OPA-01, OPA-12, OPA-16, OPA-18, OPA-20, OPC02, OPC-05 and OPH-05) were produced reproducible fragments with easily recordable bands which were selected for analysis. A total of 91 bands were detected with an average of 11.4 bands per primer. The genetic similarities were estimated from banding pattern using UPGMA clustering method. The Pearson's similarity coefficients were used to construct a UPGMA dendrogram. Pair-wise estimates of genetic similarity ranged from 0.17 to 1.00. Five primary genotypes were found within a sampling of 30 individuals: white flowers with purple markings on the bottom petal lobe (A. gangetica micrantha), purple, white, pink and yellow flowers. Our result showed the RAPD marker for assessing genetic relationship among A. gangetica that correspond to flower color.

Key words: Asystasia gangetica (Linn) T. Anderson, Randomly Amplified Polymorphic DNA (RAPD), genetic relationship

Introduction

The family Acanthaceae consists of several important medicinal plants with broad array of biological activities. *Asystasia* is the genus of Acanthaceae, with approximately 70 species that are found in tropical and subtropical old world regions (Mabberley, 1987). *Asystasia gangetica* (Linn) T. Anderson known as Chinese violet (local common names: baya, yaya) which can grow and spread quickly. This species is dispersed by seeds and rhizomes that the nodes are easily develop roots and the seeds are dispersed from explosive capsules. It has green and oval-shaped leaves with rounded base occurring in

opposite pairs. The multi-potential application of *Asystasia*, the leaves are eaten as a vegetable (Mepba *et al.*, 2007), traditional medicine and also used as an ornamental plant. *Asystasia* is known to have high nutritional value and various biologically active substances (Kanchanapoom and Ruchirawat, 2007; Hamid *et al.*, 2011; Tilloos *et al.*, 2012). Pharmacological studies, it has been claimed to have antiasthmatic, antidiabetic, anticancer and antioxidant, analgesic and antiinflammatory, antimicrobial and antifungal properties (Akah *et al*., 2003; Tilloos *et al*., 2012).

Two subspecies are recognized in Thailand (1) *A. gangetica micrantha* that flowers are mostly white in color with two parallel purple blotches lines on the bottom petal lobe and (2) *A. gangetica gangetica* that flowers are various shades color as a plum, purple, light pink, pink, white and yellow with paler to pale yellowish throats. However, genetic diversity had not been studied focusing on intraspecific in *Asystasia*. There is only a study about gametic chromosome number (n=13) and somatic chromosome number (2n=26) for *A. gangetica* in Singapore (Pandit *et al*., 2006).

Molecular techniques such as sequencing, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) are common use for taxonomical, phylogenic and genetic diversity within and between populations. In recent years, the genetic relationship among Acanthaceae has been using sequence data from the trnL-trnF region in chloroplast DNA (Mcdade and Moody, 1999). However, RAPD marker is polymorphic and evenly distributed throughout the genome, simple and inexpensive. Therefore, this technique has been widely used in genetic diversity studies in different plant (Williams *et al*., 1990; Kumar, 1999). The genetic relationship in the Acanthaceae has been assessed using RAPD markers such as *Acanthus ilicifolius* Linn. (Lakshmi *et al*., 1997), *Andrographis paniculata* (Burm. f.) Nees (Lattoo *et al*., 2008) and *Clinacanthus nutan* (Fong *et al*., 2014). The main objective of this study was to characterize the extent and pattern of genetic diversity among a collection of *Asystasia* using RAPD marker which has been applied to increase our understanding of the distribution and extent of genetic variation within and between this species.

Materials and methods

Plant materials

A. gangetica was used in this study growing in Thailand. Plant samples were collected from 10 provinces during December 2013 to February 2014 and

grown in experimental site. A detailed description of this plant is given in Table 1. Thirty plants were chosen according to their flower pattern and color. After acclimatization 2-3 young leaves were harvested fresh for DNA isolation. Six of these trees have white flowers with purple markings on the bottom petal lobe and twenty-two have various shades color with paler to pale yellowish throats (3 plum, 3 purple, 6 white, 3 light pink, 4 pink and 5 yellow flowers) that shown in figure 1.

Table 1. Numbers of *A. gangetica* from 10 provinces has been used in the study

Numbers	Provinces	Number of samples
1	Bangkok	10
2	Ratchaburi	5
3	Chachoengsao	1
4	Srisages	1
5	Khonkaen	4
6	Prachinburi	1
7	Nonthaburi	4
8	Phayao	2
9	Samutsakhon	1
10	Pathumthani	1

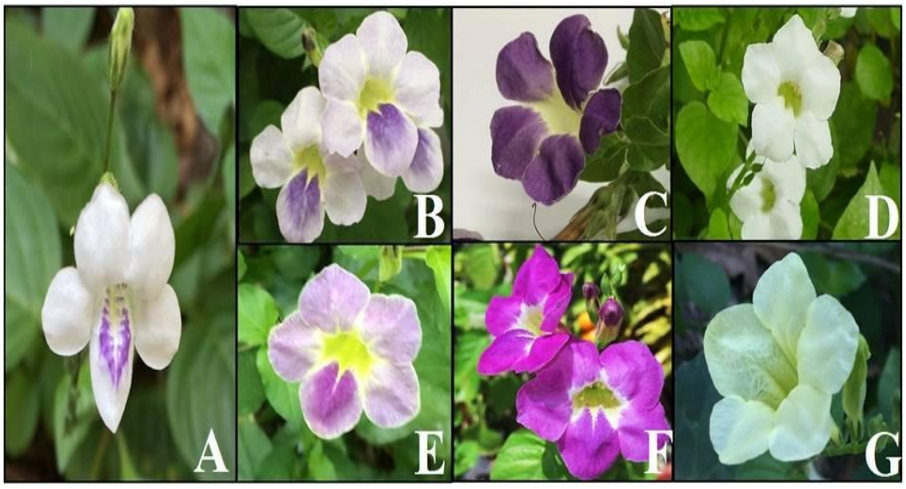

Fig 1. Flower pattern and color in *Asystasia gangetica* (Linn) T. Anderson. A: white flower with purple markings on the bottom petal lobe, B: plum, C: purple, D: white, E: light pink, F: pink and G: yellow flower color

DNA extraction

For each sample, total genomic DNA was extracted from fresh leaves by a slightly modified CTAB (Cetyl trimethyl ammonium bromide) method (Doyle and Doyle, 1987). One hundred gram of fresh leaves were frozen in liquid nitrogen and ground into a fine powder. 2XCTAB extraction buffer [2% CTAB, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 1.4M NaCl and 1% PVP] with β-mercapthoethanol was added and the mix was incubated at 65°C for 45-60 min. Equal volume of chloroform: isoamyl alcohol (24: 1) was carried out and the extract was centrifuged at 14,000 rpm/min for 5 min. After centrifugation, the supernatant was incubated with two µL of RNaseA (20 mg/mL) at 37°C for 1 hour. 10% CTAB buffer and chloroform: isoamyl alcohol (24: 1) was added and the mixture was centrifuged at 14,000 rpm/min for 5 min. Two-third volume of isopropanol was added to the supernatant and incubated at -20°C for 1 hour. DNA was collected by centrifugation at 14,000 rpm/min for 20 min and washed with 70% (v/v) ethanol. After centrifugation, the DNA was dehydrated with absolute ethanol and incubated at 37°C for 10 min. The DNA was dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Then, DNA was quantified and analyzed for quality by a spectrophotometry and agarose electrophoresis method on 1 % agarose gel in 1X TBE buffer.

RAPD analysis

RAPD analysis of genomic DNA was done using 10-mer random oligonucleotide primers (William *et al*., 1990). The extracted genomic DNA in each sample was diluted with TE buffer to a concentration of 100 ng/ µl. The twenty microliter of mixture was consisted of 100 ng of genomic DNA, 1X PCR buffer, 1.25-2 mM $MgCl_2$, 0.2 mM dNTPs, 1 pmol RAPD primer, 1 unit of *Taq* DNA polymerase and deionized water. Amplification was done using thermal cycler with following program: initial denaturation at 94°C for 1 min 45 sec, followed by 10 cycles of 1 min at 94°C, 1 min at 37°C and 1 min 45 sec at 72°C, followed by 35 cycles of 1 min at 94°C, 1 min at 56°C and 1 min 45 sec at 72°C and final extension at 72°C for 7 min 30 sec. RAPD products were separated by agarose electrophoresis on 1.5 % agarose gel in 1X TBE buffer along with 100 bp plus DNA ladder (vivantis). The gels were stained with 10 µg/ml ethidium bromide solution, examined under UV light transilluminator and photographed using gel documentation system.

Data analysis

In data scoring and analysis of RAPD: Clear and well resolved bands were compared with each other and DNA fragments were scored as a binary character (1 for presence and 0 for absence) from each primer. Coefficients of genetic similarity (GS) between the individuals were obtained using the simple matching coefficient (Rohlf, 2000) and constructed a dendrogram based on genetic similarity matrix using the UPGMA (unweighted pair group mean average) method (Sokal and Sneath, 1973) by NTSys Version 2.0e program. Including, principle coordinate analysis (PCA) among all individuals was analyzed using SPSS software.

Results and discussion

Sixty-four decamer primers were used for preliminary screening. Only eight primers (12.5%) showed reproducible fragments with easily recordable bands and gave polymorphisms. These distinguish banding patterns can be successfully and eight RAPD primers could amplify DNA from all *A. gangetica* samples. The number of RAPD bands per primer ranged from 9 (OPA-12) to 15 (OPA-18) with an average 11.4. The amplified products were then categorized based on their size ranging from 300 to 3,000 bp. In total 81 polymorphic bands were scored which OPA-18 and OPA-20 primers gave the highest number of polymorphic fragments. The 89.00 percentages of polymorphic bands were given from all of RAPD primer. A summary of eight primers sequence, amplified products and percentages of polymorphic bands from this study is shown in Table 2. Beside, the RAPD profiles generated by the primer OPA-16 are shown in Figure 2.

Table 2. Primer codes, sequences, number of amplified bands and polymorphism detected by the use of eight RAPD primers in 30 samples of *A. gangetica*

Primer codes	5'-sequence-3'	Number of amplified bands	Number of polymorphic bands	Polymorphic bands (%)
OPA-01	3' CAGGCCCTTC 5'	10	9	90.00
OPA-12	3' TCGGCGATAG 5'	9	6	66.67
OPA-16	3' AGCCAGCGAA 5'	10	9	90.00
OPA-18	3' AGGTGACCGT 5'	15	13	86.67

OPA-20	3’ GTTGCGATCC 5’	14	13	92.86
OPC-02	3’ GTGAGGCGTC 5’	12	10	83.33
OPC-05	3’ GATGACCGCC 5’	11	11	100.00
OPH-05	3’ AGTCGTCCCC 5’	10	10	100.00
	Total bands	91	81	89.00

The RAPD marker revealed by eight pairs of selected primers was used for the cluster analysis. Dendrogram constructed with the unweighted pair-group method with arithmetic mean (UPGMA) of genetic relationships among 30 samples based on simple matching coefficients varied from 0.55 to 0.95 (Figure 3) based on RAPD polymorphic data using the SIMQUAL program in the NTSYSpc software. The highest similarity was detected between the purple flowers (BY07 and BY11) and the lowest similarity was observed between BY04 and BY07/11. The thirty samples were grouped into three clusters at a 55% similarity level. The first major cluster (A) consisted of 6 samples (BY02, 04, 20, 25, 26 and 30). This cluster is belonging to *A. gangetica micrantha* that flowers are mostly white in color with two parallel purple blotches lines on the bottom petal lobe. The second major cluster was formed by 12 samples consisting of plum (B), purple (C) and white (D) flowers. The last major cluster was formed by 12 samples consisting of pink (E) and yellow (F) flowers. This resulted suggests that three subspecies will be recognized (1) *A. gangetica micrantha*, (2) the flowers are shades color as a plum, purple and white flowers with paler yellowish throats and (3) the flowers are shades color as pink and yellow with pale yellowish throats.

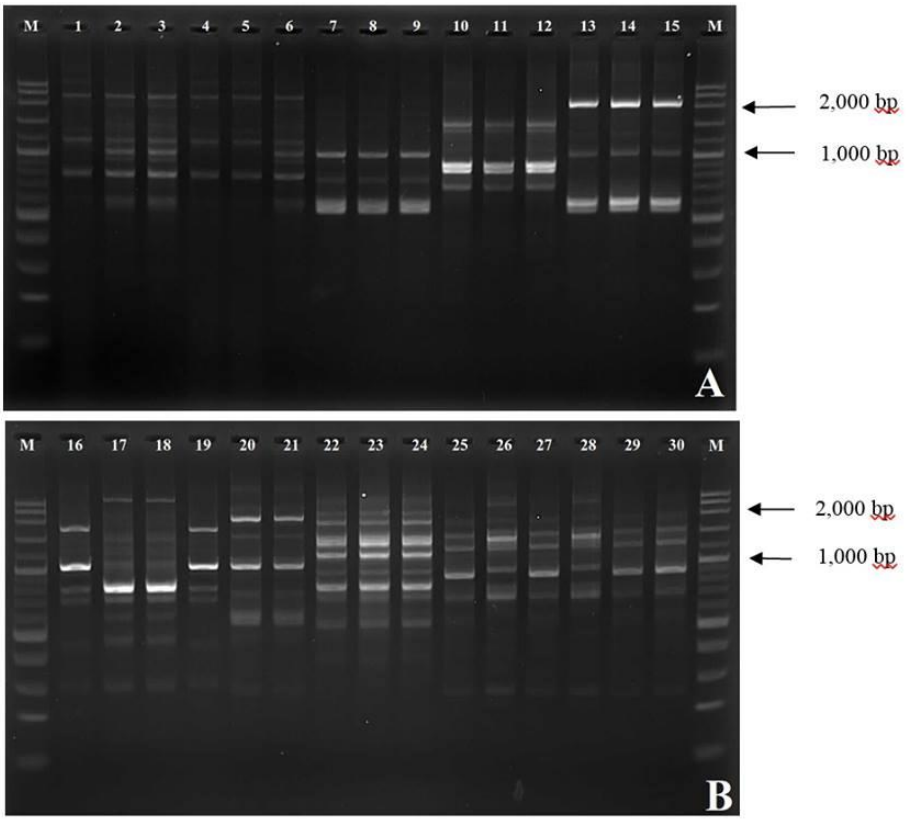

Fig. 2. RAPD profile of 30 samples of *A. gangetica* obtained with primers OPA-16, A: lanes 1-15 and B: lanes 16-30 represent samples 1-30 and M: markers (100 bp plus DNA ladder)

However, the thirty samples were grouped into six different genotypes at a 78% similarity level (Figure 3). The first genotype (A) only consisted of *A. gangetica micrantha*. Groups B, C and D were formed by 3 plum, 3 purple and 6 white flowers, respectively. Including, E and F were formed by pink and yellow flowers, respectively. This result showed the RAPD marker for assessing genetic relationship among *A. gangetica* that related with flower color.

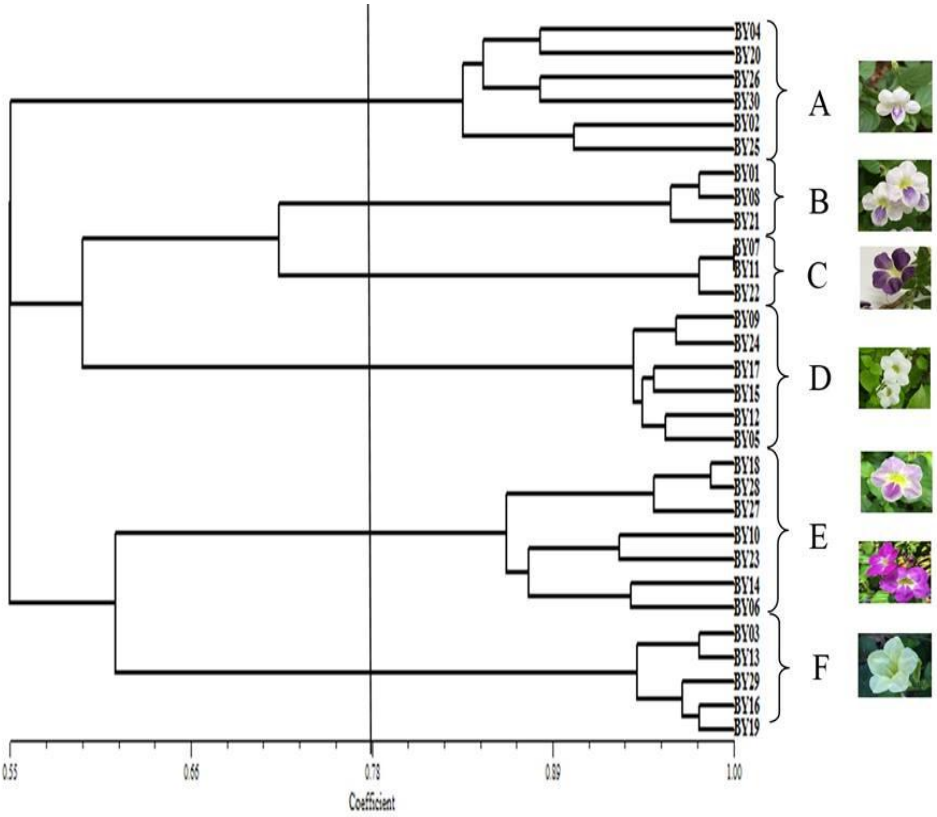

Fig 3. Dendrogram constructed with the unweighted pair-group method with arithmetic mean (UPGMA) of genetic relationships among 30 samples of *A. gangetica* based on simple matching coefficients from RAPD marker: A-F represent the six different clusters at a 78% similarity level

Conclusion

The molecular marker techniques not have been currently available for the analysis of genetic relationship in *A. gangetica*. This is first report using molecular marker which can be employed successfully for assessment of the genetic relationship of *A. gangetica* collected from different regions of Thailand. Dendrogram constructed with the UPGMA among 30 samples based on simple matching coefficients. In the present study was showed the high level of genetic diversity. Six primary genotypes were found within samplings that correspond

to flower color. However, genetic relationships of *A. gangetica* will be studied using other molecular markers to support this study.

Acknowledgement

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Diversity of endophytic fungi from orchids

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Endophyte microorganisms are those that colonize the internal tissues of healthy plants. The meaning of term "endophyte" is as broad as its literal definition and spectrum of potential hosts and inhabitants. Recently, endophytic fungi have attracted the attention from many scientists in the world. It is estimated that many endophytic fungal species may be useful as sources of anti-cancer, anti-diabetic, insecticidal and immune-suppressive compounds. In this study conducted, there were 50 isolates taken from 11 orchid varieties grown under green house condition from Rajamangala University Technology Tawan-ok Chantaburi Campus, Chantaburi Province, Thailand. Morphological identification showed that the isolates belong to 7 species of endophytic fungi namely: *Xylaria sp., Nigrospora sp., Chaetomium sp., Pestalotiopsis sp., Curvularia sp., Fusarium sp*. and *Cladosporium*-like fungi. The species which were found the most were: *Xylaria sp., Pestalotiopsis sp.* and *Cladosporium*-like fungi with percentage occurrence of 28%, 20% and 20% respectively. Moreover, *Pestalotiopsis sp., Xylaria sp.* and *Chaetomium sp.* were found to have the widest host range.

Key words: Endophytic fungi; Orchids; *Xylaria; Pestalotiopsis; Curvularia; Fusarium; Cladosporium*-like fungi

Survey of mangosteen clones with distinctive morphology in eastern of Thailand

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Mangosteen clone survey in Eastern Region of Thailand as Rayong, Chanthaburi and Trat Province in 2008 and 2009 showed diferential morphology as mangosteen phenotype was different and could be distinguished in 6 characters i.e small leave and small fruits trees, oblong shape trees, thin (not prominent) persistent stigma lobe thickness fruit trees, full and partial variegated mature leave color (combination of green and white color) trees, oblong shape leave trees and greenish yellow mature fruit color trees. Generally, rather short shoot, elliptic leaf blade shape, undulate leaf blade margin and thin or cavitied persistent stigma lobe thickness fruits are dominant marker of full seedless fruits that rarely found trees. Survey of mid-sized mangosteen orchards (200-300 trees) showed that over 70% full seedless fruits trees could be found only about 1-3% of all trees.

Key words: clones, mangosteen, phenotypes

Biodiversity of food resources in high land: A case study of Wa River at Amphur Bo Klua, Nan Province Thailand

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The research on diversity of phytoplankton, zooplankton and macroinvertebrate in Wa River, Amphur Bo Klua, Nan Province, Thailand 15 research sites along Wa River were investigated on September, 2013(wet season) and February, 2014 (dry season). A total of 37 genera of phytoplanktons; 5 genera of Cyanophyta, 19 genera of Chlorophyta and 13 genera of Chromophyta were identified. The average phytoplankton biodiversity index (H') was 1.36. A total of 6 genera/group of zooplanktons; 2 genera of Arthropoda,1 genera of Protozoa and 3 genera of rotifer were identified. The average zooplankton biodiversity index (H') was 0.38. A total of 32 family of macroinvertebrates; 8 order of Arthropoda, 1 order of Annelida and 1 order of Mollusca were identified. The average macroinvertebrate biodiversity index (H') was 1.59. Diversity of phytoplankton, zooplankton and macroinvertebrate were changed by seasonal and geographic characteristics of the region. The result of this research provides basic knowledge about biodiversity, especially, high land for management area for conservation area.

Key words: Phytoplankton, Zooplankton , Benthic fauna, Species diversity, River

Structure and distribution of demersal fish in Moo-KhoBulon, Satun Province, Thailand

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Structure and distribution of demersal fish in Mo KhoBulon, Satun Province. From April 2012 - March 2013, but the fishing boat survey was only 5 months is May 2012 June 2012 July 2012 January 2013 and March 2013 because the climate is not conducive to fishing this year is fishing the least of which. Compared to several years ago. The composition of species Mo Kho Bulon, Satun Province. The study found there are various species up to 69 species of fish, 64 species groups are 2 species of groups, squid, crab, crayfish 3 species, grasshoppers only 1 species. Dominants Species is found on the island of Mo Kho Bulon, Satun Province by number Species is found *Sillago sihama* of 1,112 characters (representing 55.54%), followed by *Netuma thalassinus* 104 characters (equivalent to 5.19%), sand, white sandfish *Scolopsis taenioptera* the number 83 (or 4.15%), *Upeneus luzonius* of 79 characters (equivalent to 3.95%), respectively. Dominants Species is found on the island of Mo Kho Bulon, Satun Province by weight.Species is found on the island of Mo Kho Bulon, Satun Province by weight were found that fish with *Sillago sihama* total weight of 40,955.70 grams (equivalent to 52.39%), followed by fish spines *Netuma thalassinus* total weight of 6268.60 grams (equivalent. 8.02%), white fish *Scolopsis taenioptera*total weight of3707.80grams (equivalent to 4.74%), respectively. *Sillago sihama* total weight of 40,955.70 grams (equivalent to 52.39%), followed by fish spines *Netuma thalassinus* total weight of 6268.60grams (equivalent. 8.02%), Saitama, sand fish, white fish *Scolopsis taenioptera* total weight of 3707.80 grams (equivalent to 4.74%), respectively. Standing Crop with an average of 125.07 kg. per hectare. By most in the month of July2012 was 200.05 kg. per hectare. And lowest in May was 45.19 kg. per ha. The catch per unit effort with nets caught fish (CPUE) from this study at the fishery on average 6 hours showed an effect catch average of 2.61 kg. per hour. The study found that The index species (richness index) of the month of July2012 with a variety of species, most, followed by the June 2012 January 2013 March 2013 and May 2012 respectively. The island of Mo Kho Bulon, Satun Province. Average water quality the salinity of the water was 30 ppt, pH was7.8 and the average DO was 5.9 mg / L average water depth of 12 meters and an average water temperature of 30 ° C.

Key words: Species diversity of fish, Species composition, Abundance,Dominants Species, Standing Crop,

Genetic assessment of the genus *Charadrius* based on *ATPase 6/8* gene sequences

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Global climate change provide critical habitat for migratory shorebirds. Genus *Charadrius* are migratory shorebirds in Thailand during non breeding season. The morphological features used to identify species of this genus. However, it is difficult because there does not appear to be different. So, the objectives of this study were (i) used *ATPase 6/8* gene sequences to determine species and (ii) to define the phylogenetic relationship among *Charadrius* sp. The plover were from 3 areas: Samut Sakhon Province (Central), Phetchaburi Province (West) and Trang Province (South of Thailand) by cannon-nets. Genetic differences between populations were assessed using partial of *ATPase 6/8* sequence data from the mitochondrial DNA. The phylogenetic tree can be divided into three clades including Lesser Sand Plover (Charadrius mongolus), Greater Sand Plover (C. leschenaultii) and Alexandrinus complex plover. The Alexandrinus complex plover showed monophyly which can be subdivided into three species: Kentish Plover (C. alexandrinus), White-faced Plover (C. alexandrinus dealbatus) and Malaysian Plover (C. peronii). Thus, sequence of *ATPase 6/8* gene helpful identifies species in Lesser and Greater Sand Plover that the ambiguity identified in the field. Accordingly this study, the result of genetic base will also have implications for migratory shorebird in Thailand.

Keywords: *Charadrius*, Plover, *ATPase subunit 6/8* genes, Phylogenectic tree

Species composition and abundance of penaeid shrimps in the outer Songkhla Lake of Thailand

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The coastal waters of Songkhla Lake in Thailand constitute an important habitat for penaeid shrimps. Despite the high economic value attached to this resource, the biological information necessary for its sustainable exploitation is scanty and fragmented. The present study was therefore designed to investigate the species diversity composition of the penaeid shrimps in Songkhla Lake. Samples were obtained monthly for (during January 2010 to Janauary, 2011) a period of thirteen month from Outer Songkhla lake, samples were identified to the species level. The samples were collected using trap nets. Twenty-two species from 6 genera of Penaeidae were reported from the present study. Six species of *Penaeus* (Farbricius, 1798) were found; *P. japonicus* (Bate, 1888), *P. latisulcatus* (Kishinouye, 1896), *P. monodon* (Fabricius, 1798), *P. semisulcatus* (De Haan, 1844), *P. merguiensis*(De Man, 1888) and *P. silasi* (Muthu and Motoh, 1979). There were two speciesof the genus *Metapenaeopsis* (Bonvier, 1909)*; M. stridulans* (Alcock, 1905) and *Metapenaeopsisbarbata* (De Haan, 1844). The only species in genus *Solenocera* (H. Milne Edwards, 1837) was *Solenocera crassicornis* (H. Milne Edwards, 1837). There were 7 species of *Metapenaeus*(Wood-Masen, Alcock, 1891); *M. lysianassa*(De Man, 1888), *M. intermedius* (Kishinouye, 1900), *M. ensis* (De Haan, 1844), *M. affinis* (H. Milne Edwards, 1837), *M. moyebi* (Kishinouye, 1896), *M. brevicornis* (H. Milne Edwards, 1837) and *M. tenuipes* (Kubo, 1949). There were 3 species of *Parapenaeopsis* (Alcock, 1901); *P. hungerfordii* (Alcock, 1905) *P. hardwickii* (Meirs, 1878) *P. sculptilis* (Heller, 1862) The genus *Trachypenaeus* (Alcock,1901) were found 3 species. There were 3 species of *Trachypenaeus* (Alcock, 1901); *T. sedili* (Hall, 1961), *T. malaiana* (Balss, 1933) and *T. pescadoreensis* (Schmitt,1931). The species composition of Penaeid shrimps were divided three seasons; rainy season, summer season and interseason. The most dominant Penaeid shrimps species were *M. moyebi*, *M. ensis*, *P. silasi* and *P. merguiensis*throughout the entire study period. In general, *Penaeus* spp. and *Metapenaeus* spp. were the most abundant species found in the Outer Songkhla Lake and season. Shrimp juveniles move out from the Songkhla Lake into the Gulf of Thailand. The species composition and population abundance in this study is a fundamental development of a management policy for modeling of a shrimp population in Outer Songkhla Lake.

Key words: Penaeidae, Marine shrimp, Species composition, Abundance, outer SongkhlaLake

Efficiency for enzyme production of fungi isolated from the stomach of buffalo

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A study on the efficiency for enzyme production of fungi isolated from stomach of buffalo was conducted. The fungi were collected from 4 parts of stomach as rumen, reticulum, omasum and abomasums. The objective to study the efficiency of fungi from stomach of buffalo had effected to produced enzyme and to selected fungi for their ability to produced enzyme cellulase, hemicellulase and ligninase. Results shown that the fungi isolated from rumen were : *Eupenicillium* sp. (B-RU-01-1), *Eupenicillium* sp. (B-RU-02-3G), *Rhyzopus stolonifer* (B-RU-01-4) and *Trichoderma* sp. (B-RU-01-2). From the reticulum, *Aspergillus glaucus* (B-RET-02-3), *Aspergillus orchraceus* (B-RET-02-2) and *Penicillium* sp. (B-RET-02-4) were found. In the omasum *Aspergillus fumigatus* (B-OMA-01-1G), *Eurotium* sp. (B-OMA-01-4) and *Rhizopus stolonifer* (B-OMA-02-3) were isolated and in the abomasums *Aspergillus flavas* (B-ABO-02-3), *Aspergillus fumigatus* (B-ABO-02-1), *Aspergillus niger* (B-ABO-01-3G), *Aspergillius terreus* (B-ABO-02-4) and *Mucor* sp. (B-ABO-02-4G). Results of enzyme analysis revealed that cellulase was produced by isolated : *Eupenicillium* sp. (B-RU-02-3G), *Eupenicillium* sp. (B-RU-01-1), *Penicillium* sp. (B-RET-02-4), *Aspergillius glaucus* (B-RET-02-3), *Aspergillus ochraceus* (B-RET-02-2), *Aspergillius fumigatus* (B-OMA-01-1G), *Eurotium* sp. (B-OMA-01-4), *Aspergillius flavus* (B-ABO-02-3), *Aspergillius fumigatus* (B-ABO-02-1), *Aspergillius niger* (B-ABO-01-3G), *Aspergillius terreus* (B-ABO-02-4). Hemicellulase was produced *Eupenicillium* sp. (B-RU-02-3G), *Eupenicillium* sp. (B-RU-01-1), *Rhizopus stolonifer* (B-RU-01-4), *Trichoderma* sp. (B-RU-01-2), *Aspergillius glaucus* (B-RET-02-3), *Aspergillus ochraceus* (B-RET-02-2), *Penicillium* sp. (B-RET-02-4), *Aspergillius fumigatus* (B-OMA-01-1G), *Eurotium* sp. (B-OMA -01-4), *Aspergillius flavus* (B-ABO-02-3), *Aspergillius fumigatus* (B-ABO-02-1) *Aspergillius niger* (B-ABO-01-3G), *Aspergillius terreus* (B-ABO-02-4), *Mucor* sp. (B-ABO-02-4G). For the enzyme ligninase ,two isolates were found to produced this enzyme namely : *Trichoderma* sp. (B-RU-01-2) and *Mucor* sp. (B-ABO-02-4G).

Key words: enzyme production from fungi, enzyme production

The *Piriformospora indica*, Magic Fungus and Its Role on Sustainable Agriculture

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The conversion of plant production systems from conventional resource-exhausting to sustainable systems depends on management of environmental factors. Root-inhabiting fungi are considered as a main factor since their hyphae connect in ideal manner and challenge of the surrounding with the plant. One of these most important root endophytic fungi is

Piriformospora indica. This fungus has a broad host spectrum and positively affects different aspects of plant performance. *P. indica* has multifunctional activities like plant growth promoter, bio-fertilizer, immune-modulator, bio-herbicide, phytoremediator, etc. Effect of *P. indica* has been studies on more than 150 plants. Promising outputs of laboratory experiments and small field trials indicated the need for its mass cultivation and usage. *P. indica* has proved to be highly beneficial endophytic fungus with high efficacy in filed.

Key Words: *Piriformospora indica*, Sebacinales, Root Endophytic Fungi, Plant growth promotion.

SESSION 3: Organic Agriculture and Biological Control

Reduced incidence of tomato yellow leaf curl virus and leafminer in a tomato cultivar in northern Thailand

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Tomato leaf curl virus (TYLCV) is a whitefly-vectored begomovirus that is an important limiting factor for tomato production worldwide and causes extensive losses to the crop in Thailand. Although many TYLCV-resistant tomato cvs. have been developed worldwide, field evaluation of commercial tomato cvs. for resistance to the virus endemic in northern Thailand has not been reported. Two experiments were conducted in Lampang, northern Thailand in 2011-2012 and 2012-2013 which compared the field performance of three tomato cvs. from the U.S.A., Christmas Grape, Husky Cherry Red, and Roma, with Seeda, a commonly used Thai cv. in experiment one, and Husky Cherry Red with Seeda in experiment two. Plant characteristics evaluated included yield, and disease, insect and nematode resistance. The experiments used minimal plant management inputs; dried cow manure was incorporated into the soil prior to planting, followed by biweekly application of liquid fish fertilizer, and irrigation as necessary. No pesticides of any kind were used in either experiment. There were few significant differences observed among the cvs. in marketable yield, and root- knot (*Meloidogyne* sp.) and root rot (*Rhizoctonia solani*, *Fusarium* spp. and *Sclerotium rolfsii*) severity. However there were significant differences noted in TYLCV incidence; incidence of the virus ranged from 38-100% and 8-41% in experiments one and two, respectively. In both experiments 'Husky Cherry Red' had the lowest (p<0.05) TYLCV incidence, leafminer damage (*Liriomyza* sp.), and progression of foliar necrosis severity. The foliar necrosis was presumably caused by a combination of leaf miner and thrips (*Ceratothripoides claratris*) feeding, and air pollution from annual forest and other fires in the region. TYLCV infection was confirmed by a simplified method of nuclear inclusion visualization with light microscopy in experiment one, and by virus inclusion detection and PCR in experiment two.

Key words: virus, leaf curl, tomato, leafminer

Innovative healthcare products derived from organic farming

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A World Health Organization survey indicated that about 70–80% of the world populations rely on non-conventional medicine mainly of herbal sources for the treatments of common health problems. In the recent years, there has also been an increasing growth in the market of health foods, nutraceuticals, and medicinal products from plants or other natural sources in developed countries. . Such increase in popularity has also brought concerns and fears over the quality,

efficacy and safety of the products. Over the past decade adverse effects, sometimes life threatening, have been reported from taking OTC herbal products or traditional medicines from various ethnic groups. These OTC products may be contaminated with excessive or banned pesticides, microbial contaminants, heavy metals, chemical toxins, and for adulterated with drugs. The risk of contamination is much greaer, if they are grown under contaminated environment. Some of these environment related factors can be controlled by implementing standard operating procedures (SOP) leading to Good Agricultural Practice (GAP), Good Laboratory Practice (GLP), Good Supply Practice (GSP) and Good Manufacturing Practice (GMP) for producing these medicinal products from herbal or natural sources. Organically grown food is grown and processed using no synthetic fertilizers or pesticides. Plant medicines possess the unique ability of regeneration. In response to growing demand for herbal and natural products our group have always believed that the whole plant has a much greater value than any single extracted material. Using medicinal mushrooms, as example, this paper illustrates how organic farming and advances in biomedical analysis would help to minimize toxic contaminants in herbal substances. Medicinal mushrooms and mushroom extracts are used to fight cancers and enhance immune system. Mushrooms have also been scientifically proved to having anti-bacterial and anti-viral properties. The Chinese and the Egyptians were the first to recognize the health benefits of mushroom. Both Chinese and Egyptians believed that mushroom can promote longevity and included mushroom as a specialty in the diet of the royal family. Today, the mushroom is part of expensive cuisines in luxurious restaurants worldwide. It is also used as active ingredients for modern medicine. More extensive research on health benefits of medicinal mushroom by ways of clinical trials should be encouraged. Medicinal fungi are those fungi that can produce unique metabolites having medical benefits. The range of these medically active compounds include antibiotics, antifungals, anti-cancer drugs, cholesterol lowering substances, psychotropic drugs and immunosuppressants. Pharmacological research has confirmed that many forms and phyla of fungi including mushrooms have a long history of medicinal uses. Ganoderma lucidum, ling zhi, is probably the fungus with the longest record of medicinal benefits. Many edible species have been shown to produce medically significant metabolites. However, most compounds of interest when being used for medical purposes need to be synthesized on an industrial scale and are administered in ways that maximize the potential benefits. About 100 species of mushrooms are currently being studied for their health benefits. Mushrooms are known to concentrate heavy metals, as well as air and water pollutants, so healthy growing conditions is a critical factor. At present, we are in the process of formulating and manufacturing active metabolites extracted from *Cordyceps sinensis* and its related species in order to obtain several products having scientifically-proven efficacy.

Key words: organic products, mushroom

Advanced research and development for organic crop production

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Microbial products have been developed as agricultural inputs for organic crop production over 25 years. These are now successfully are used to reduce damage and to decrease toxic chemicals and applied in commercial scale of organic farms. These microbial products are as follows:- microbial fertilizer, Ketomium as biofungicide, nano-elicitor for plant immunity and bioinsecticide. These bioproducts have been applied either for organic crop production or good agricultural practice in Thailand, Laos, Vietnam, Malaysia, Myanmar and Cambodia. Successful applications in the field trials have also been demonstrated in several countries, e.g. P.R. China, Taiwan, Costa Rica, Philippines, Bangladesh, Georgia and Russia. Organic crop production has been increasingly interested in several countries. It is still needed to search for new agricultural inputs to be applied for organic farms. The new biological fungicide would be released for control diseases. The detail information will be discussed in the conference.

Keywords: microbial products, Organic crop production

Biological control of rice brown leaf spot caused *Curvularia lunata* and application method in the field of rice variety IR66 in Cambodia

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Curvularia lunata was found to be caused brown leaf spot of rice var. IR66 planted in Cambodia. It becomes one of the most commonly encountered blight diseases caused by *C. lunata* in Cambodia. The symptoms were observed from leaves, spots and blight were brown in color. It is a first reported of blight disease of rice in Cambodia. All isolates were proved for pathogenicity. Bi-culture antagonistic test showed that *Chaetomium cupreum* could be inhibited sporulation of *C. lunata* at 28.55 % when compared to the control plate. *Ch. cupreum* can be significantly reduced disease incidence of brown leaf spot caused by *C. lunata* in pot experiment after application of spore suspension of *Ch. cupreum,*biofungicide (ketomium) and chemical fungicide (tebuconasole) to inoculated rice seedlings with C. lunata can be reduced disease of 83.96, 87.92 and 86.75 %, respectively. In the field trial, chemical method gave the best result in all plant parameter and followed by GAP method and organic method. Chemical method gave better in panicle/plant, panicle length (g), panicle weight(g), grain weight(g)/plant which significantly differed when compared to GAP and organic method. Chemical method

gave the best result in filled grain/panicle, unfilled grain/panicle, grain weight (kg)/plot, dry hay weight(kg)/plot, bio mass weight(kg)/plot and Harvest Index (5%) significantly better than GAP and organic method.

Key words: rice brown leaf spot, rice, *Chaetomium* sp

Biological activity of endophytic fungi associated with palm trees

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Endophytic fungi - the fungus that live within plant tissues and without causing any symptoms - were isolated from leaf, petiole and root of palm trees in King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand, and used to biological activity test. A total of 60 pure isolates were obtained by morphological identification and *Phoma exigua.*, *Fusarium chamydosporum*, *Phialophola spp*. and *Nigrospora spp.* were examined for bi-culture antagonist test against *colletotrichum coffeanum* from coffee Anthracnose. The result showed that all of these 4 species endophytic fungi gived the antifungal activity. And *Nigrospora spp*. gived the best inhibition, which was 5.67cm in colony growth , 8.75×10^5 in spore numbers and 69.72% in the inhibition percentage of spore production.

Key words: Endophytic fungi; palm trees; *Colletotrichum coffeanum*; bi-culture antagonist test

Metabolites from *Chaetomium* spp inhibit *Colletotrichum gloeosporiodes* causing anthracnose on citrus

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Biomasses of *Chaetomium cupreum* and *Chaetomium lucknowense* were extracted with hexane, ethyl acetat and methanol to obtain crude hexane, EtAOc and MeOH, respectively from each fungus. The crude extracts then were tested at different concentrations for inhibition of mycelial growth and spore formation of *Colletotrichum gloeosporiodes* which causing anthracnose on pomelo (*Citrus maxima*). The results showed that at 1,000 µg/ml, the hexane, ethyl acetat and methanol extract from *Ch.cupreum* respectively inhibited 44.25, 51.45 and 54.75 % of mycelial growth and 85.65, 92.6, 97.45% of spore production of *C. gloeosporiodes* with ED50 was 452, 155 and 114 µg/ml (respectively). At concentration of 10 µg/ml all three crude extracts from *Ch. lucknowense* showed significant inhibition of mycelial growth of *C. gloeosporiodes*, and gave the highest inhibitory rates of 77.25, 82.63 and 78.50% at 1,000 µg/ml by the hexane, ethyl acetat and methanol extract, respectively. All these crude extracts inhibited completely spore production of the pathogen (100%) at 1,000µg/ml, and their ED50 valued of 80, 46 and 32 µg/ml, respectively. Crude extracts *Ch. lucknowense* showed stronger inhibitory effect on both mycelia growth and spore production of *Colletotrichum* sp than the same type crudes of *Ch.cupreum*.

Key words: metabolites, anthracnose, crude extract

Application of *Chaetomium cupreum* for biocontrolof *Colletotrichum coffeanum* causing coffee anthracnose in Arabica variety in Laos

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Coffee anthracnose pathogen was isolated from leaves and coffee bean lesions and identified as *Colletotrichumcoffeanum*, then proved for pathogenicity. Bi-culture antagonistic test expressed *Chaetomumcupreum* inhibited colony growth and spore production of *C. coffeanum*as 29 and 38 %, respectively. Bioactive substances as hexane crude extract, ethyl acetate crude extract and methanol crude extract from *Ch. cupreum* showed good efficacy to inhibited *C. coffeanum* which rhe ED50 values were 13, 11 and 28 ppm. Pot experiment showed that Chaetomium-biofubgicide significantly decreased disease incidence of 69 % and followed by nano-cg, nano-trichotoxin and spore suspension of *Ch. cupreum* 57, 56 and 49 %, respectively when compared to the non-treated control. It is the first report on application of *Ch. cupreum* for biocontrolof *C. coffeanum* causing coffee anthracnose in Arabica variety in Laos

Key words: coffee anthracnose, Arabica variety

Epidemiological situation of maize downy mildew in Thailand

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Five species of maize downy mildew fungi including *Peronosclerospora sorghi* (Sorghum downy mildew), *P. spontanea* (Spontaneum downy mildew), *P. philippinensis* (Philippine downy mildew), *P. sacchari* (Sugarcane downy mildew) and *Sclerophthora rayssiae* var. *zeae* (Brown stripe downy mildew) have been reported in Thailand. However, all species were identified and reported based on morphological characteristics. The aim of this study was to clarify the fungal species and physiological race of maize downy mildew distributed in Thailand. The downy mildew was surveyed and estimated the disease severity in maize plantation of Thailand. The disease samples were collected to the laboratory for morphological and physiological race observation. The results showed that there was high disease severity on sweet corn and waxy corn with more than 70% occurrence. All fungal isolates were large, hyaline and long conidiophores with dichotomously branched, hyaline and oval to almost spherical conidia. The septum in basal conidiophore was observed and used as the distinctive morphological character of *P. sorghi*. The differential varieties of maize were inoculated with downy mildew fungi for race identification. The results indicated that twenty five isolates of *P. sorghi* were identified as five races. However, the species of downy mildew fungi in Thailand will be identified and supported by the molecular data.

Key word: *Peronosclerospora sorghi*, downy mildew, maize, corn, physiological race

Evaluation of two species of Trichoderma as compost activator and bio-control agents

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The two *Trichoderma* species were found to be viable in improving the composting efficiency of the compost especially when the *Trichoderma* sp. 2 was inoculated on the compost. *Trichoderma* sp. 2 increased the total NPK content of the compost at 4.88% with the C: N ratio of 6:1. Combination of the two species of *Trichoderma* could also increase the nutrient content of the compost with 4.48% with the high percentage recovery of 67%. However, compost inoculated with *Trichoderma* sp. 1 had the lowest total NPK content of 3.68% as compared with the compost not inoculated with activator (3.93%). The two *Trichoderma* species had the ability to inhibit the mycelia growth of *S. rolfsii* under in-vitro condition by 34.92% and 31.44%, respectively. Different mycoparasitic activity was observed among the two species of *Trichoderma* and this could be attributed to the released of different metabolites against *S. rolfsii* The two *Trichoderma* were able to produce metabolites, by inhibiting the growth of *S. rolfsii* by 10.46% to 29.03% in volatile, 3.74% to 5.59% in non-volatile, and 11.3% to 12.94% in direct-diffusible metabolite. Biological activity of *Trichoderma* on seed coating treatment showed enhancement of the seed germination of cucumber when the seeds were coated with *Trichoderma* sp. 1 (93.75%) alone, or coated with *Trichoderma* sp. 1 + *Trichoderma* sp. 2 (84.38%). Seeds not coated with any of the *Trichoderma* increased the percentage of disease incidence as compared with the seeds coated with *Trichoderma* either alone or in combination. Biological activity of the *Trichoderma* on soil treatment revealed that seeds sowed in any of the infected or non-infected soil treated with the two species of *Trichoderma* either alone or in combination enhanced the seed germination of cucumber by 87.50% to 93.75%. Disease incidence in cucumber was high even in the presence of *Trichoderma* sp. 1; however application of the two species of *Trichoderma* reduced the incidence of damping off disease by 6.25%. *Trichoderma* species were found effective as compost activator in rice straw-based compost as it enhance the nutrient content of the compost. Likewise it produce different amount of metabolites that inhibit the growth of *S. rolfsii*. The two species of *Trichoderma* also helped to improve seed germination and reduce the disease incidence of cucumber in seed and soil treatment application.

Key words: *Trichoderma*, compost activator, bio-control agent

Testing nanomaterials derived from *Trichoderma harzianum* to control *Colletotrichum capsici*

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Chili production is one of the most profitable crop in Thailand due to the high demand for it. However, the production of chili is affected by some diseases like anthracnose. This research was conducted to determine the effect of nanomaterial containing methanol extract from *Trichoderma harzianum*. Results show that in terms of colony diameter at 9 days of incubation, the use of pure compound has 4 cm which was significantly higher than all treatments.

However, The use of 5 ppm and 10 ppm nanomaterial containing methanol crude extract produced 4.26 cm and 4.18 cm respectively and do not vary significantly from each other. .Spore production was observed to be significantly different among treatments. Abnormal spores were also observed in the treatments using crude extracts but more were observed in the treatments using nanomaterial.

Key words: chili, anthracnose, nanomaterial

Application of *Myrothecium roridum* as a biocontrol agent for controlling water hyacinth

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Myrothecium roridum is a leaf blight disease pathogen of water hyacinth. The purpose of this study to evaluate the application of *M. roridum* for controlling water hyacinth in natural water resource. The effective strain was applied by spore suspension with surfactant such as Tween 20 and palm oil. Fungal spore suspension was added with 1% Tween 20 or 10% palm oil, then it was sprayed onto water hyacinth leaves under natural condition. The result indicated that the disease severity was increasing when mixed spore suspension with Tween 20 or oil palm. Moreover, crude extracts of fungal mycelia in hexane, ethyl acetate and methanol solvents were evaluated on water hyacinth leaves. The result showed the leaf blight occurs on water hyacinth leaves after treated with crude extracts. The results indicated that mycotoxins might be released from fungal mycelia and caused the leaf blight on water hyacinth.

Key words: leaf blight disease pathogen, disease severity, crude extracts

Initial results of the study antagonistic fungi *Chaetomium globosum* to control impotant diseases on tea *Camellia sinensis* in Vietnam

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Tea plant *Camellia sinensis* is not only a popular but also an important income with people who live in midlands and mountainous areas. Because of diseases and insects, it's more and more difficult for people to plant and produce tea. Now aday, tea disease is becoming more compicate and most of the farmers only use chemical pesticides to prevent disease which cause environment polluted and affect to producers and consumers's health. *Chaetomium globosum* which is isolated in Phu Tho Province and activity evaluated with fungal disease on tea such as anthracnos (*C. camelliae)* and root rot (*Fusarium sp.),* has prevent mycelium development from 57,11% to 75% and spores development of 2 species of diseases from 58,73% to 77,38%. It also limited the spread of *C. camelliae* when directly treated on tea leaf. These results has paved the way for applying *Chaetomium* on preventing tea disease effectively and safety.

Key words*: Camellia sinensis, Chaetomium globosum, Colletotrichum camelliae, Fusarium sp.,* antifungal activity.

SESSION 4: Biotechnology and Natural Products

Secondary metabolites from *Chaetomium* spp. and mushroom

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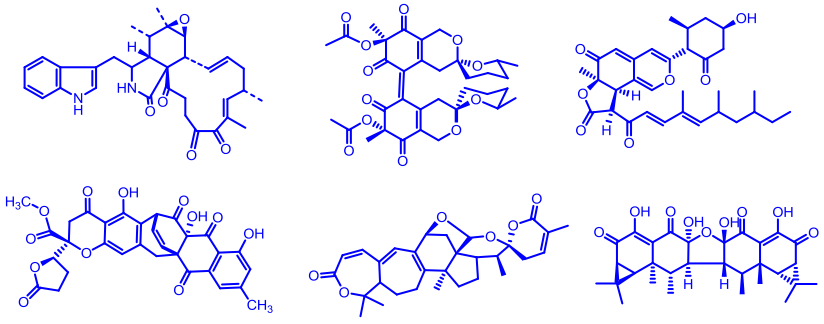
Investigation on fungi isolated from Thai soil genus *Chaetomuim* spp, and mushrooms, *Scleroderma citrinum*, *Neonothopanus nambi* and *Ganoderma* sp. KM01 resulted in the isolation of numerous types of compounds for example cytochalasans, xanthones, xanthoquinodins, azaphilones, depsidones, and terpenoids. The isolated compounds were evaluated for their bioactivities such as antimalarial activity against *Plasmodium falciparum*, antimycobacterial activity against *Mycobacterium tuberculosis*, as well as cytotoxicity against KB, BC1, NCI-H187 and cholangiocarcinoma cell lines. This presentation will highlight our works on chemical and biological aspects of these isolated compounds.



Key words: *Chaetomuim*, *Scleroderma citrinum*, *Neonothopanus nambi*, *Ganoderma* azaphilone, terpenoid, antimalarial, anti-TB, cytotoxic

Chemical constituents from some saprophytic fungi

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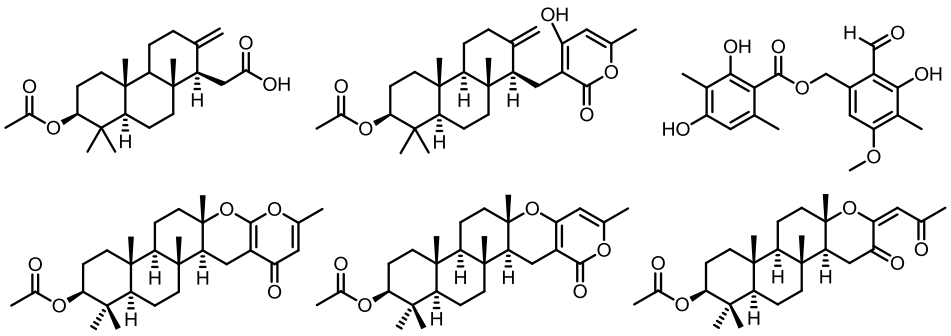
Investigation on saprophytic fungi isolated from Thai soil genus *Eurotuim chevalieri*, *Neosartorya tatenoi* KKU-2NK23 and *Gymnoascus reessii* resulted in the isolation of meroterpenoids and benzyl ester. These isolated compounds were evaluated for their bioactivities such as antimalarial activity against *Plasmodium falciparum*, antimycobacterial activity against *Mycobacterium tuberculosis*, and cytotoxicity toward three cancer cell lines, KB, BC1, and NCI-H187. This presentation will highlight our works on chemical and biological aspects of these isolated compounds.



Key words: *Eurotuim chevalieri*, *Neosartorya tatenoi*, *Gymnoascus reessii*, meroterpenoid, antimalarial, cytotoxic

Two edible mushrooms' interaction against fusarium wilt which caused by F. oxysporum f. sp. lycopersici

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The majority of mushrooms are edible, medicinal or health care value, development value is high. The two collected specimens Clitocybe spp AJ2-2, Boletus affinis var. maculosus AJ2-3 were from the rain forest which located in Kanchanaburi Province, Amphoe Mueang Kanchanaburi ,Thailand (N 14°0′12″, E 99°33′0″). Crude extracts were yielded from the two specimens. Results showed that the crude hexane, crude ethyly acetate and crude methanol from Clitocybe spp AJ2-2 yielded 5.92, 5.48 and 5.99%, respectively. The crude hexane, crude

ethyly acetate and crude methanol from B. affinis var. maculosus AJ2-3 yielded 0.43, 0.47 and 5.32 %, respectively. The crude extarcts from Clitocybe sp AJ2-2 and B. affinis var. maculosus AJ2-3 were selected for bioactivity test against fusarium wilt which caused by F. oxysporum f. sp. lycopersici. Result showed that crude ethyly acetate from Clitocybe sp AJ2-2 gave significantly highest inhibition of 83.90 % for spore production of F.oxysporum at concentration of 1000 ppm. Crude hexane from B. affinis var. maculosus AJ2-3 gave significantly highest inhibition of 76.91 % for the spore production ofF.oxysporum at the concentration of 1000 ppm. These investigations are also reported for the first that time that Clitocybe, B. affinis var. maculosus and have shown some antimicrobial substances against fusarium wilt which canused by F.oxysporum. Further investigation would be studies on chemical elucidation of these antagonistic substances.

Key words: mushroom, chemical constituents, bioassay

Antifungal substances from *Chaetomium cupreum* against *Pestalotia* spp. Causing gray blight disease of tea

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Tea is one of the most popular and lowest cost beverages in the world, next only to water. Tea trees are planted in 46 countries all over the world which range from Mediterranean-type climate to the hot humid tropics. Diseases on tea are very various infected. This research was focused on grey blight disease of tea caused by *Pestalotia* spp. Hexane, EtOAc and MeOH crude extract from *Chaetomium cupreum* were used to test antimicrobial substance against *Pestalotia* spp. The results showed that *Ch.cupreum* significantly inhibited *Pestalotia* spp. with the ED50 of 28.40 -154.12 µg/ml.

Key words: tea, antimicrobial substance test, *Pestalotia* spp., *Chaetomium cupreum*,

Cytotoxicity and antimicrobial activities of leaf extracts from *Barleria strigosa*

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Our preliminary phytochemical screening demonstrated that the methanolic extract from leaves of *Barleria strigosa* had in vitro cytotoxicity against the P-388 murine leukemia cell line with CC_{50} (50% cytotoxicity concentration) of 413.89 µg/ml and exhibited strong antibacterial activity against *Bacillus subtilis* with an inhibition zones of 14 mm at 10 mg/disc. Therefore, this present study attempts to evaluate the cytotoxicity and antimicrobial activity. The crude methanol extract was subjected to a liquid-liquid partition yielding hexane, dichloromethane, ethyl acetate and butanol extracts described by Beedessee *et al.,* (2012). The cytotoxicity of all extracts were analyzed using MTT colorimetric assay described by Mosmann (1983) against six cancer cell lines (HepG-2, MCF-7, KB, HT-29, P388 and Hela cells) and two normal cell lines (Vero and L929 cells) at concentration range of 62.5 to 1000 µg/ml. By the MTT assay, the strongest cytotoxic activity was obtained from the butanol extract followed by the ethyl acetate, dichloromethane and hexane extract, respectively. Highest cytotoxicity of butanolic extract was found against P-388 cell line (CC_{50} = 127.42 µg/ml) and found cytotoxic against L-929, KB, Hela, MCF-7, HT-29, Vero and HepG-2 cells at the CC_{50} levels of 283.00, 287.22, 566.83, 574.19, 666.78, 835.92 and >1,000 µg/ml, respectively. The extracts were tested for their antimicrobial activity against five pathogenic bacteria (*Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Staphyloccus aureus*) at concentration range of 0.125 to 2 mg/disc using paper disc diffusion method described by Ansari *et al.,* (2005). The butanol extract exhibited more interesting antimicrobial activity than other extracts, being especially active against *M. luteus* and *S. aureus* at 2 mg/disc with diameters of the inhibition zones was 8.8 and 8.22 mm, respectively. The results indicated that butanol extract exhibited cytotoxicity and antimicrobial activities. Therefore, the fractions from butanolic extract were also appreciating for further investigations in future.

Key words: Cytotoxicity, Antimicrobial activity, *Barleria strigosa*, MTT colorimetric assay, Paper disc diffusion method

Introduction

Thailand is one of the countries that have a variety of biological resources. Many of the members of the Acanthaceae family are used as medicinal plants and have been reported in the scientific literature to possess promising bioactive compound such as *Thunbergia laurifolia* (Tangpong and Satarug, 2010), *Andrographis paniculata* (Kumar *et al*., 2004) and

Rhinacanthus nasutus (Puttarak *et al*., 2010). Bioactive compound screening is very important in identifying new sources phytochemicals that might lead to drug development. Genus *Barleria* is member of Acanthaceae which are herbs and widely distributed throughout tropical Asia. The bioactive compounds and biologically active extracts have been reported from this genus. Especially in *Barleria lupulina*, leaves, stem, root and flower of *B. prionitis* possess antibacterial and anti-inflammatory activities (Singh *et al*., 2003; Amoo *et al*., 2009; Jaiswal *et al*., 2010; Chavan *et al*., 2010; Shukal *et al*., 2011). The methanol extract of leaves and stems had a pronounced blood-glucose-lowering potential in in diabetic animals (Suba *et al*., 2004; Dher and Bhatnagar, 2010). Verma *et al*. (2005) found methanolic extract of this plant to produce anti–spermatogenic effect without affecting the general body metabolism. Iridoid enriched fraction of aerial parts was demonstrated for hepatoprotective activity in various acute and chronic animal (Singh *et al*., 2005). Bark extract is effective in controlling candidiasis and other oral fungal infections (Aneja *et al*., 2010) and antioxidant property (Ata *et al*., 2009). In *B. cristata*, the ethanol extract from seeds of *B. cristata* had the effect to reduce the blood sugar of mice (Singh *et al*., 2012). In others, the extracts from various parts of *B. prionitis, B. greenii, B. albostellata* had the antibacterial, antifungal and anti-inflammatory (Amoo *et al*., 2009; 2011). Dichloromethane extract of *B. argillicola* had a stronger antibacterial activity against the gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) than gram-positive bacteria (*Staphylococcus aureus*) (Amoo *et al*., 2013).

As previously discussed found the plants in *Barleria* had demonstrated for the biological activity. Despite the popular use *B. strigosa* as a medicinal plant, there are no data about the biological activity. The aim of the present study was to evaluate the potential cytotoxicity and antimicrobial activity of different extracts obtains hexane, dichloromethane, ethyl acetate and butanol derived from *B. strigosa* using MTT colorimetric assay and paper disc diffusion method, respectively. Including, the chemical composition were analyzed by the GC-MS.

Materials and methods

Plant material

Characteristics of *B. strigosa* are shown in Fig. 1. The leaves of *B. strigosa* were collected in June 2013 from the Eastern Botanical Graden (Khao Hin Son), Chachoengsao, Thailand. The samples were rinsed thoroughly in

running tap water to remove soil particles and adhered debris. The leaves were dried in an oven at 45°C for 3-4 days and ground in coarse powder as well as stored in a plastic bag.

Fig.1 Plant of *Barleria strigosa*

Preparation of extract

1 kg powder of dried leaves was macerated with 95% methanol at room temperature on shaker for 3 times of 7 days. After maceration, the extracts was filtered using Whatman filter paper No.1 and evaporated to dryness on a rotary evaporator under reduced pressure at 40◦C and removed the last tract solvent in desiccator. This constituted the crude extract, which was dissolved in distilled water to be partitioned subsequently with hexane, dichloromethane, ethyl acetate and butanol to afford of the level of polarity described by Beedessee *et al.* (2012) Finally, the extracts were weighed and stored in universal bottles and refrigerated at 4°C prior to use used.

Cell cultures

The six cancer cell lines including human hepatocellular carcinoma (HepG2), human breast adenocarcinoma (MCF7), human oral epidermoid carcinoma (KB), human colon adenocarcinoma (HT29), murine lymphocytic leukemia (P388) and human cervical carcinoma (HeLa) as well as two normal cell lines including african green monkey kidney (Vero) and mouse subcutaneous connective tissue (L929) were used for cytotoxicity assays (Fig.2). The cells were cultured in RPMI-1640 medium with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 0.1% gentamycin and were grown at 37 °C in 5% CO_2-humidified atmosphere in 25 cm^2 flasks. When the cells reached 80%

confluence, they were washed with phosphate-buffered saline (PBS) and harvested with 1 ml of 0.25% trypsin-EDTA solution (except P388).

MTT colorimetric assay

In vitro cytotoxic activity has been measured using modified MTT assay described by Mosmann (1983). Briefly, viable cells were counted by trypan blue exclusion using a haemocytometer (Freshny, 1994). The cell lines (100 µl/ well) were seeded at seeding densities about 1-1.5×10^5 cells/ml into 96-well plates and incubated for 24 h at 37°C. After this incubation period, the cells were treated with different extracts (hexane, dichloromethane, ethyl acetate and butanol) at various concentrations. The highest concentration of the extracts (2000 µg/ml) was added to row 1 of the 96-well plates and several 2-fold serial dilutions were made until row 5. The 0.2% (v/v) dimethyl sulphoxide (DMSO) and 0.5% anticancer drug mitomycin C (MMC) were used as negative and positive control, respectively. After incubated for 24 h, MTT (2mg/ml in PBS) were added and incubated for 4 h at 37 ◦C. The media was carefully removed with a needle and syringe. The formazan crystals were dissolved in 100 µl DMSO: ethanol (1: 1) and the absorbance was measured at 570 nm using a microplate reader. All the concentrations were tested in triplicated on the same cell batch.

Microbial strains and culture condition

Five microbial strains: *B. subtilis* ATCC6633, *E. coli* DMST4212, *M.luteus* TISTR9341, *Psu.aeruginosa* ATCC27853 and *S.aureus* TISTR1466 were used for antimicrobial activity. All microorganisms were cultured in Mueller-Hinton Broth (MHB) and incubated at 37°C for 24 h. To adjust the concentration of suspensions was O.D. 0.5-0.8 at 600 nm.

Paper disc diffusion

The antimicrobial activity of plant extracts were tested by using agar disc diffusion described by Ansari *et al.* (2005). Each bacterial suspension was swab over the surface of Mueller Hinton Agar (MHA) with a sterile cotton swab. Various concentrations of extracts (0.125, 0.25, 0.5, 1 and 2 mg/disc) were dissolved in methanol and loaded into the 6 mm sterile paper discs, then were placed on the agar. The same volume of methanol was used a negative control and gentamicin was used as positive control due to its broad spectrum of activity against various organisms. After being kept at room temperature for 30 min,

they were incubated at 37°C for 24 h. The diameters (mm) of the inhibition zone were measured and the presented values are average of three separate experiments.

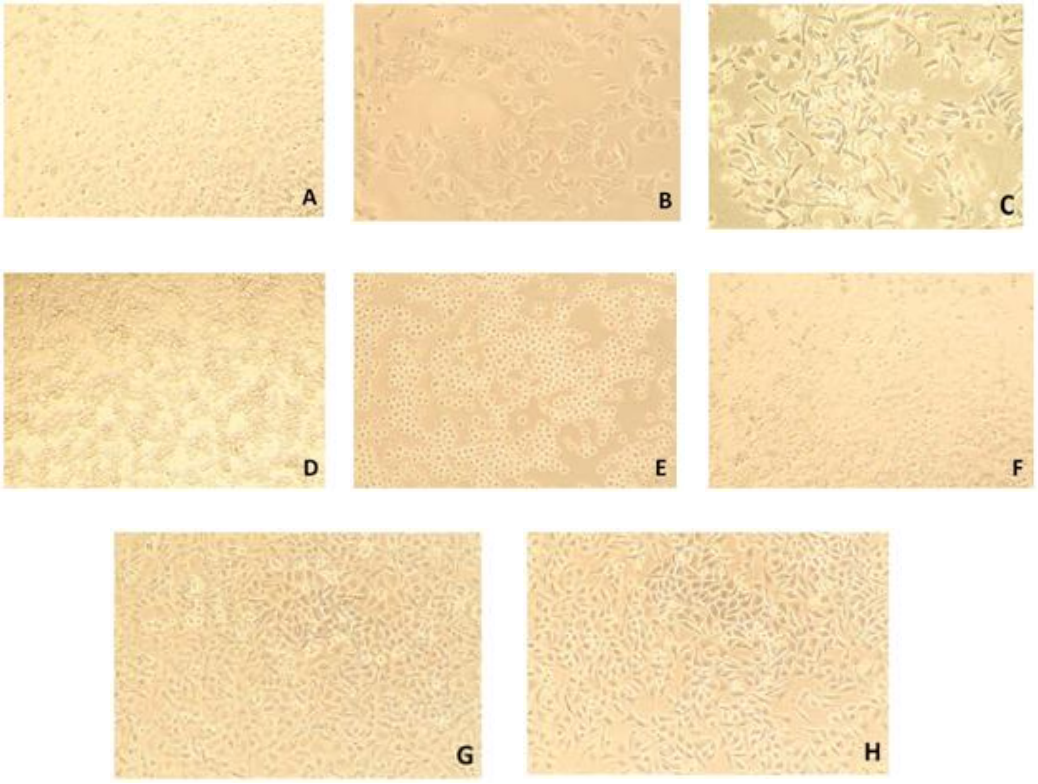

Fig.2 Morphological appearance of the cell lines: (A) human hepatocellular carcinoma (HepG2), (B) human breast adenocarcinoma (MCF7), (C) human oral epidermoid carcinoma (KB), (D) human colon adenocarcinoma (HT29), (E) murine lymphocytic leukemia (P388), (F) human cervical carcinoma (HeLa), (G) african green monkey kidney (Vero) and (H) mouse subcutaneous connective tissue (L929)

Gas chromatography-mass spectrometry (GC-MS)

The extracts were sent to analyzed by the GC-MS using Acq method at Scientific Instruments Center, KMITL. An aliquot of 10:1 was injected (split) into the GC-MS . The data were obtain on a HP-5 column (30 m x 0.25 mm, 0.25 µm). The carrier gas was helium 99.99% and the GC oven temperatrue program was as follows: 70°C hold for 5 min, rate of 8°C/min to 290°C and hold for 12 min. The

injector and detector temperatures were set at 250°C and the pressure were 8.73 psi. The mass range was scanned from 50 to 500 amu.

Statistical analysis

Experiments were performed in triplicate and the results were expressed by using GraphPad Prism5 and Microsoft Excel 2007.

Results and discussion

MTT colorimetric assay

The cytotoxicity of all extracts were analyzed using MTT colorimetric assay in eight cell lines (L929, KB, Vero, HeLa, HepG-2, HT-29, P-388 and MCF-7) at concentration ranging from 62.5-1000 µg/ml. The result in the hexane extract was found the CC_{50} against to Vero and L929 cell lines were 763.1 and 934.7 µg/ml, respectively. The dichloromethane extract was found the CC_{50} against to L929, P-388 and MCF-7 were 687.68, 728.07 and 960.14 µg/ml, respectively. The ethyl acetate extract was found the CC_{50} against to P-388, L929, HeLa, KB and HT-29 were 654.27, 932.09, 944.83, 985.41 and 981.73 µg/ml, respectively. The butanol extract was found the CC_{50} against to P-388, L929, KB, HeLa, MCF-7, HT-29 and Vero were 127.42, 283.00, 287.22, 566.83, 574.19, 666.78 and 835.92 µg/ml, respectively (Fig.3). The research of the our preliminary of methanolic extract from leaves of *B. strigosa* had in vitro cytotoxicity against the P-388 murine leukemia cell line with CC_{50} of 413.89 µg/ml (Manapradit *et al*., 2013) and not found the previous report on cytotoxicity about this plant.

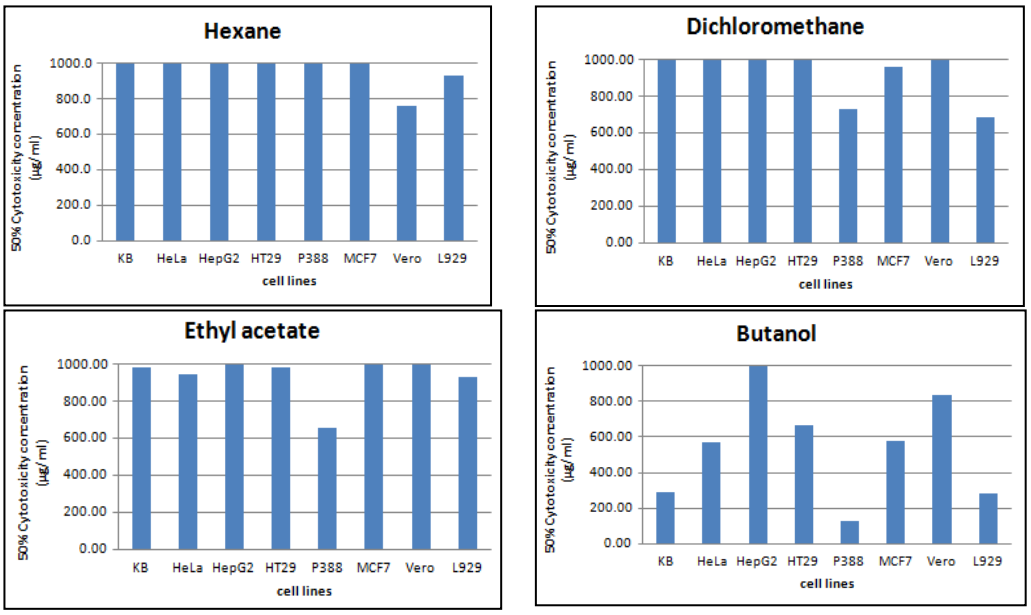

Fig.3 The 50% cytotoxicity concentration (CC_{50}) of eight cell lines after the addition of the hexane, dichloromethane, ethyl acetate and butanol extract at various concentrations were analyzed using MTT colorimetric assay.

Paper disc diffusion

All extracts were tested for their antimicrobial activity against five pathogenic bacteria strains (*Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Staphyloccus aureus*) at concentrations ranging from 0.125-2 mg/disc using paper disc diffusion method. The results was found the butanol extract had active against for *M. luteus* and *S. aureus* at 2 mg/disc and the diameters of the inhibition zones was 8.8 and 8.22 mm. respectively (fig.4). The hexane, dichloromethane and ethyl acetate extracts was not found to be active against any of the test strains at this various concentrations. The researches of the other plant in Barleria genus were showed antibacterial activity as the petroleum ether extract from *B. prionitis* at concentration 10 mg/ml had maximum antibacterial activity againt *Klexsiella pneumonia* (Chavan *et al*., 2010). Including, Salib *et al*. (2013) found the ethanolic extract from *B. cristata* at concentration 10 mg/ml was strong inhibitory activity against *S. aureus*, *B. subtillis* and *S. mutans*.

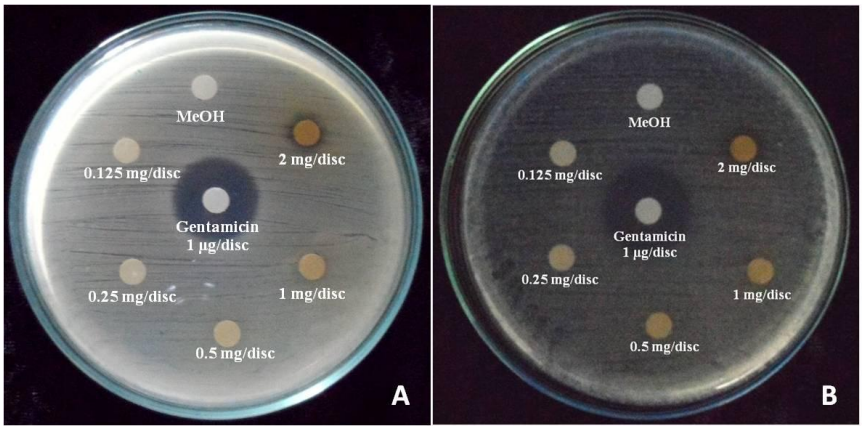

Fig.4 The diameters of the inhibition zones of butanol extract: (A) *S. aureus* and (B) *M. luteus* at concentrations ranging from 0.125-2 mg/disc using paper disc diffusion method.

Gas chromatography-mass spectrometry (GC-MS)

The extracts were analyzed by GC-MS system. The results of the hexane, dichloromethane, ethyl acetate and butanol extract found 13, 28, 26 and 22 compounds, respectively. The main chemical compositions of different extracts were presented in Table 1. The extract in each partition has a different chemical composition and not found the previous report study about the chemical composition by GC-MS in this plant. However, the other plant in *Barleria* sp. were studies about chemical constituents such as Ata *et al.* (2009) were analyzed structures of the compound from the aerial parts of *B. prionitis* by NMR spectral and found the one new phenylethanoid glycoside, barlerinoside along with six known iridoid glycosides, shanzhiside methyl ester, 6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydiderroside, and lupulinoside. Including, Salib *et al.* (2013) were analyzed the structures of the compounds from the ethanolic extracts of the *B. cristata* bark by UV spectral and found the new flavonoid compound 6-O-α-L-rhamnopyranoside-3,7,3'-O-trimethylated-8-hydroxyquercetin and identified for the first time in nature together with the known flavonoids 6-O-α-L-rhamnopyranoside quercetagetin, 3-methoxy quercetin, gossypetin 8-methyl ether, quercetagetin, tamarixetin, gossypetin and quercetin.

Table 1. The main chemical composition from the different extracts of *B. strigosa*

Extracts	Compounds	Main chemical composition	Relative amount (%)
Hexane	13	9,12,15-octadecatrien-1-ol	18.746
		9-octadecenoic acid	14.814
		Phytol	12.795
Dichloromethane	28	2(4H)-benzofuranone	40.167
		ethyl ester	9.992
		Cyclopentane	5.856
Ethyl acetate	26	ethyl ester	17.918
		4-butylbenzonitrile	17.479
		benzoic acid	11.803
Butanol	22	m-tolunitrile	16.581
		Benzonitrile	11.970
		cyclohexane acetic acid	10.304

Conclusion

The present study demonstrated the pharmacological potential of *B. strigosa*. The cytotoxicity and antimicrobial activities of *B. strigosa* was reported for the first time. No previous report on the biological activity of this plant. The butanol extract was found the greater effect of their cytotoxicity and antimicrobial activity. The chemical composition was found the highest chemical in dichloromethane extract were 2(4H)-benzofuranone but the butanol extract were found the most of m-tolunitrile. The results provided evidence that the studied plant extracts might be potential sources of new anticancer and antibacterial drug and the butanol extract is suitable to be used to study in further and suggests the therapeutic potential of this plant when foraged by animals.

Acknowledgment

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Isolation and identification of Trichocomaceae from soil by morphology and DNA sequencing

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Abstract: The Trichocomaceae contains some of the most familiar fungi, such as *Penicillium* and *Aspergillus*. This family is cosmopolitan in distribution, ubiquitous in soil communities and extremely common associates of decaying plant material and foodstuffs. The objectives of this work were to isolate and identify varieties of Trichocomaceae mainly based on the morphological and molecular characteristics. The Trichocomaceae were isolated from soil sample were collected from Chiang Mai's forest, Thailand by soil plate technique. These fungi have been partially characterized using morphological traits such as features of colony morphology, size and shape of conidia that were grown on potato dextrose agar (PDA) at 25°C. The ITS regions, β-tubulin gene and camodulin gene were amplified using the primers that was a unique fragment of approximately 550, 450 and 650 bp, respectively. The nucleotide sequences demonstrate the level of genetic diversity of Trichocomaceae and related to the two genera: *Talaromyces flavus* (EU02, EU03, EU07, EU12 and EU14), *Talaromyces trachyspermus* (EU10 and EU23), *Neosartorya hiratsukae* (EU06) and *Neosartorya pseudofischeri* (EU13). However, a new classification system including both anamorph and teleomorph species will be investigating the relationship of some genera of Trichocomaceae.

Key words: Trichocomaceae, *Talaromyces*, *Neosartorya*

Introduction

The Trichocomaceae is a large family of well-known fungi for their advantage and disadvantage. They are associated with food spoilage and mycotoxin production and can occur in the indoor environment. The most well-known species of this family belongs to the genera *Aspergillus*, *Penicillium* and *Paecilomyces*. Species belonging to Trichocomaceae are predominantly saprobic and represent some of the most catabolically and anabolically diverse microorganisms are known (Houbraken and Samson, 2011). It belongs to Ascomycota which is the largest phylum of fungi with over 64,000 species (Kirk *et al*., 2008). Ascomycota which do not have sexual stage to form asci and ascospores, previously placed to Deuteromycota with asexual stage or anamorph which are now identified based on morphology and phylogeny analyses of DNA sequences. Ascomycota have been grouped of absence of asci. Sexual and asexual isolates of the same species commonly carry different

binomial species names, for example: *Aspergillus nidulans* for asexual and *Emericella nidulans* for sexual isolates of the same species (Alexopoulos *et al*., 1996). Asexual reproduction is the dominant form of Ascomycota which occurs through vegetative reproductive spores namely conidia. Trichocomaceae is a widespread and abundant which are known as asexually reproducing fungi. This asexual stage is interested because it implies to maintain competitive edge without the benefits of genetic recombination. Some mycologists have argued that these fungi do have sexual stages but do not know how to find them. Others believe that there are no sexual stages and so many Trichocomaceae are large amount of genetic diversity through mutations themselves. The relatives in genera of Trichocomaceae are still unclear according to the single-name nomenclature (Houbraken and Samson, 2011).

Trichocomaceae is an excellent presentation with a key, illustrations, discussions and descriptions of the species commonly found on foods and indoor environments (http://website.nbm-mnb.ca/mycologywebpages/NaturalHistoryOfFungi/Eurotiales.html)**.** Trichocomaceace is identified as higher classification known as Eurotiales. Teleomorphs of *Aspergillus* species belong to different genera in Trichocomaceae, Eurotiales, Eurotiomycetes. Many species of *Aspergillus* are known to reproduce sexually, producing asci and ascospores. The asci are nearly spherical and borne in nearly spherical cleistothecia. The cleistothecia may themselves be borne within stromatic tissues that range from simple masses of hülle cells to hard sclerotium-like structures. These are discussed in more detail in the section dealing with Eurotiales, Phylum Ascomycota (Webster and Webster, 2007; Yazdani *et al*., 2011). This research finding was to identify soil fungi based on morphological and three regions DNA sequencing.

Materials and methods

Samples collection

The soil samples were collected from Doi Suthep and Doi Inthanon mountains (Chiang Mai, Thailand) in May 2012. All samples were kept in sealed plastic bags and brought to laboratory at Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The samples were originally isolated by soil plate technique according to the method described by Soytong (1992).

Fungus isolation

The Trichocomaceae fungi were isolated from soil sample by soil plate technique using glucose ammonium nitrate agar (GANA: glucose 20 g, NH_4NO_3 1 g, difco bacto yeast extract 1 g, K_2HPO_4 0.5g, rose bengal 0.06 g, $MgSO_4$ $7H_2O$ 0.05g, agar 20 g in 1L distilled water). Soil samples were ground into a powder and put onto GANA media, then incubated for 15 days at room temperature (27-30 °C). Fungi growing out as a colony was transferred to potato dextrose agar (PDA) to get pure culture and maintained on PDA for the duration of the experiment.

Morphological identification

The Trichocomaceae fungi were grown on PDA for 10-15 days at 25ºC. Colony characters and microscope feature were observed. Each species was identified based on the methods of Domsch et al. (1993) and Soytong (1992).

DNA extraction, amplification and sequencing

All Trichocomaceae fungal colonies were cultured on potato dextrose broth (PDB) for 10-15 days at 25ºC. Fungal genomic DNA was obtained from the mycelia of the PDB cultures using the GF-1 plant DNA extraction kit (vivantis, USA).

The ITS ribosomal DNA regions was amplified by PCR using the universal primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC) (White *et al*., 1990). The *β*-tubulin gene was amplified using Bt2a (5′-GGTAACCAAATCGGTGCTGCTTTC) and Bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC) (Hubka and Kolarik, 2012). The calmodulin gene was amplified with primers CF1L (5'-GCCGACTCTTTGACYGARGAR) and CF4 (5'-TTTYTGCATCATRAGYTGGAC) (Peterson, 2008). Twenty-five microliter PCR reaction mixture contained 50 ng of DNA template, 0.8 pM of each primer, 200 µM dNTPs, 1x buffer, 1 unit of *Taq* DNA polymerase. PCR condition for the ITS regions were programed as follows: initial denaturation at 95 ºC for 1 min, followed by 35 cycles of denaturation at 95 ºC for 1 min, annealing at 50,52 ºC for 1 min, and extension at 72 ºC for 2 min, and final extension at 72 ºC for 5 min. PCR amplification for *β*-tubulin gene and calmodulin gene were performed using the conditions which comprised 32 cycles under the following temperature regime: 1 cycle of 95 °C/3 min,

55 °C/30 sec and 72 °C/1 min, follow by 32 cycles of 95 °C/30 sec, 55 °C/30 sec and 72 °C/1 min and final cycle of 95 °C/30 sec, 55 °C/30 sec and 72 °C/10 min (Hubka and Kolarik, 2012). The PCR products were purified with PCR purified kit. Sequencing was performed at First Base Laboratories, Malaysia.

Molecular phylogenetic analysis

The nucleotide sequences were conducted by comparing the DNA sequences against those available in the NCBI Genbank database using a BLASTn search. The DNA sequences were aligned using BioEdit program and performed maximum parsimony in MEGA5. Bootstrap value was determined using heuristic searches with 1000 replications.

Results and discussions

Isolation and morphological identification

In this study, nine isolates showed slow growing property with pigment on PDA at 25 °C. The morphological study was observed as microscopic characters which these isolates were shown two different groups of characters in colony, cleistothecia, asci and ascospores (Fig. 1-2) as reported by Domsch and Gams (1993). Nine isolates coded were morphologically compared as shown in Table 1. Seven isolates (EU02, EU03, EU07, EU10, EU12, EU14 and EU23) were showed yellow colony, ascomatal walls composed of loose hyphae, ascospores are broadly ellipsoidal to ellipsoidal; mostly 1.5~3.5 x 2.5~5.3 µm. Two isolates (EU06 and EU13) were showed white colony, ascospores are subglobose to broadly ellipsoidal with furrow and ridges. Therefore, EU02, EU03, EU07, EU10, EU12, EU14 and EU23 are identified as *Talaromyces* sp. EU06 and EU13 are identified as *Neosartorya* sp.

Table 1. Morphological characters of isolated Trichocomaceae

Code	Colony Color	Cleistothecia (µm)	Asci (µm)	Ascospore (µm)
EU02	yellow			
- shape		subglobose	subglobose	broadly ellipsoidal
- size		200.66~332.05 x 260.75~406.83	7.32~11.44 x 7.95~11.93	1.13~2.91 x 1.92~3.35
EU03	yellow			
- shape		globose	subglobose	ellipsoidal
- size		154.79~261.76 x 160.81~231.39	7.61~8.89 x 8.12~9.16	2.14~3.44 x 2.14~4.56
EU06	white			
- shape		globose	globose	broadly ellipsoidal
- size		90.7~263.05 x 91.98~276.76	9.86~13.63 x 8.63~13.97	3.86~6.81 x 3.70~6.48
EU07	yellow			
- shape		subglobose	globose	ellipsoidal
- size		220.78~420.59 x 244.4~482.06	8.51~9.95 x 8.69~11.69	2.91~4.42 x 4.27~5.46
EU10	yellow			
- shape		subglobose	subglobose	ellipsoidal
- size		266.63~429.15 x 221.18~404.67	7.1 1~9.01 x 5.36~8.00	2.05~4.11 x 2.04~3.92
EU12	yellow			
- shape		subglobose	subglobose	broadly ellipsoidal
- size		219.28~769.80 x 219.95~867.84	8.19~11.18 x 9.32~12.17	2.60~4.84 x 2.95~5.30
EU13	white			
- shape		globose	subglobose	subglobose
- size		151.70~458.70 x 148.06~526.58	10.33~14.19 x 10.63~15.87	4.22~6.07 x 4.49~7.41
EU14	yellow			
- shape		subglobose	globose	broadly ellipsoidal
- size		161.15~377.71 x 172.48~408.21	6.31~10.45 x 8.38~11.46	2.79~3.93 x 3.27~5.27
EU23	yellow			
- shape		globose	subglobose	ellipsoidal
- size		401.62~763.75 x 401.91~892.28	5.63~7.76 x 5.85~9.54	2.09~3.92 x 2.40~4.37

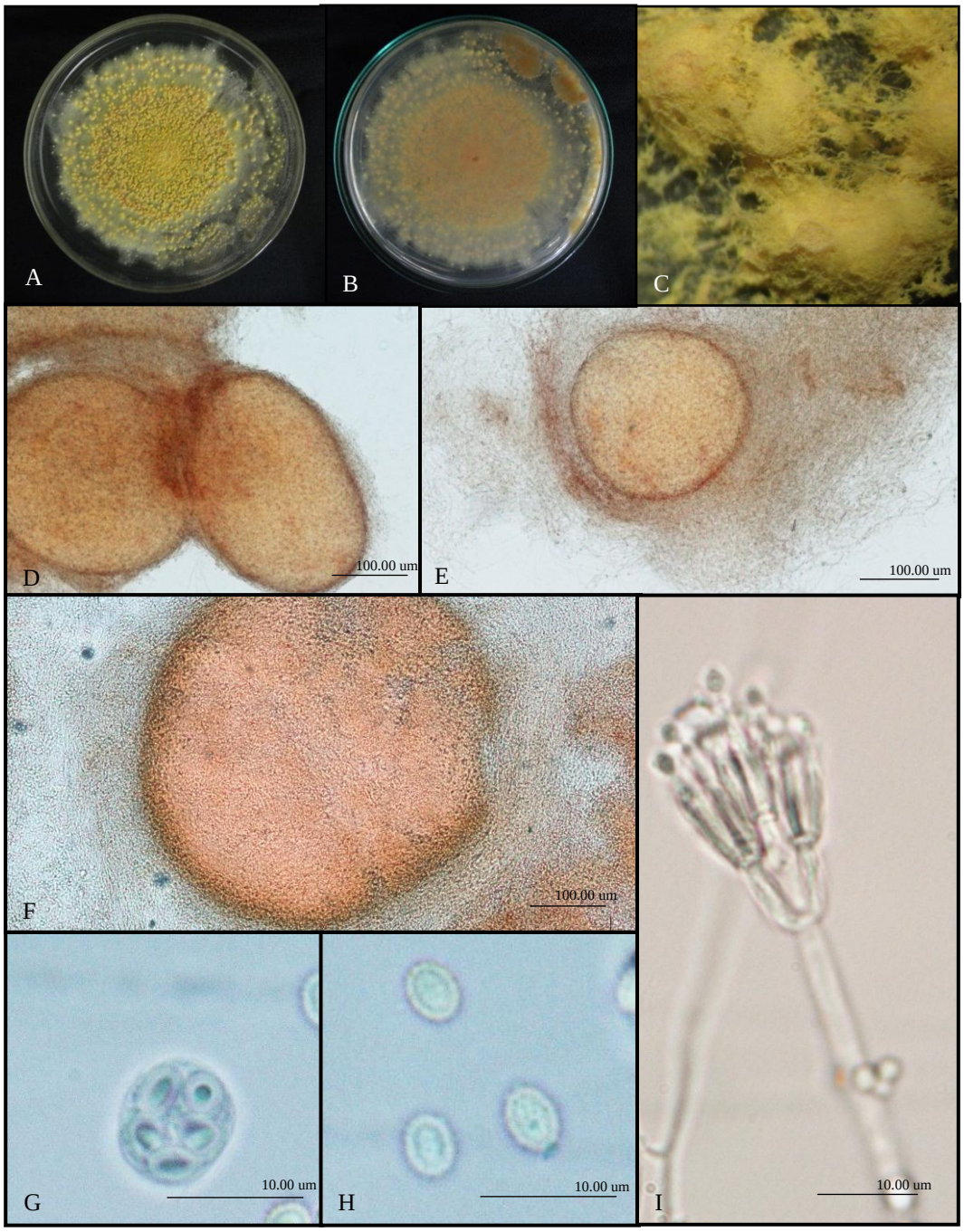

Fig. 1 Morphological features of *Talaromyces* sp. (EU12) A-B: Colony grown for 15 day at 25 °C, C-E: Cleistothecia, F: Asci exposed from cleistothecia, G: Ascus, H: Ascospores and I: Thallus, phialophores and phialides

Fig. 2 Morphological features of *Neosartorya* sp. (EU06) A-B: Colony grown for 15 day at 25 °C, C-E: Cleistothecia, F: Asci exposed from cleistothecium, G: Ascus and H: Ascospores

DNA sequencing identification

The nucleotide sequences were compared with BLASTn in NCBI Genbank demonstrated the level of genetic diversity of Trichocomaceae (Table

2) that related to the two genera. The *Talaromyces* sp. group is identified as *T. flavus* (EU02, EU03, EU07, EU12 and EU14) and *T. trachyspermus* (EU10 and EU23). The *Neosartorya* sp. group is identified as *N. hiratsukae* (EU06) and *N. pseudofischeri* (EU13).

Table 2. The three regions of nucleotide sequences compared with BLASTn in NCBI Genbank

Code	ITS regions	β-tubulin gene	Calmodulin gene
EU02	*P. verruculosum*	*T. marneffei*	*T. flavus*
EU03	*P. verruculosum*	*T. marneffei*	*T. flavus*
EU06	*N. hiratsukae*	*N. hiratsukae*	*N. hiratsukae*
EU07	*P. verruculosum*	*T. marneffei*	*T. flavus*
EU10	*T. assiutensis*	*T. trachyspermus*	*T. trachyspermus*
EU12	*P. verruculosum*	*T. marneffei*	*T. flavus*
EU13	*N. pseudofischeri*	*N. pseudofischeri*	*N. pseudofischeri*
EU14	*P. verruculosum*	*T. marneffei*	*T. flavus*
EU23	*T. trachyspermus*	*T. trachyspermus*	*T. trachyspermus*

The nucleotide sequences from the ITS regions confirmed all isolates identified as the Trichocomaceae. The three region: ITS, *β*-tubulin and calmodulin were amplified at 550, 450 and 650 bp fragments in each isolate, respectively. Maximum parsimony tree of three regions of Trichcocomaceae were represented the same relatives with the sample from NCBI-based to confirm the species. The phylogenic tree of calmodulin gene and the three regions were demonstated representative of this group (Fig. 3-4).

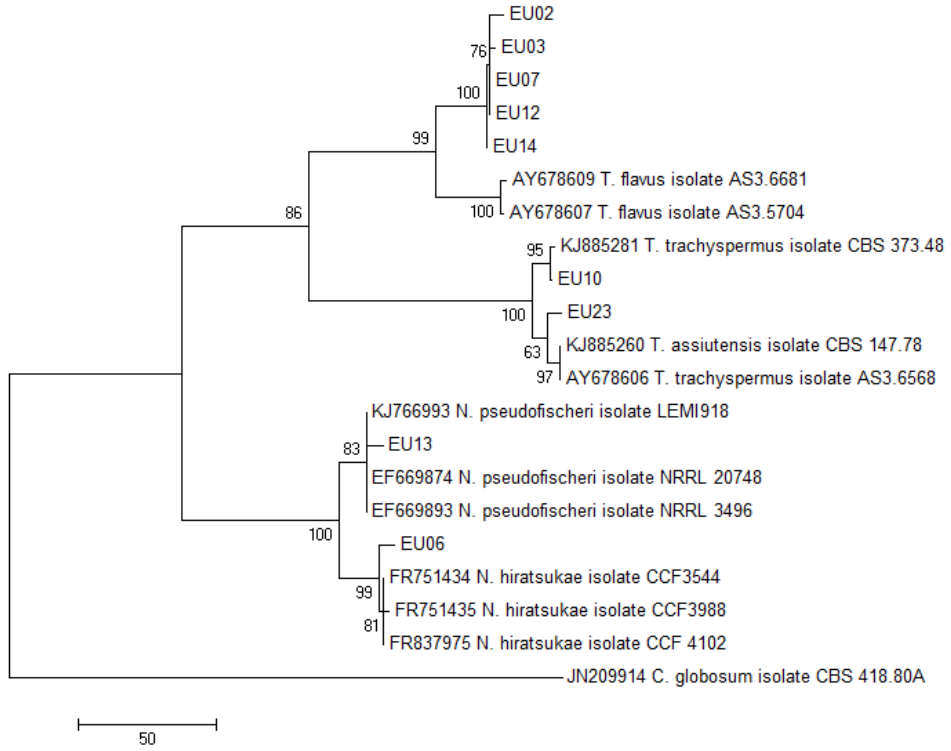

Fig. 3 Maximum parsimony tree of some Trichocomaceae based on calmodulin gene using *Chaetomium globosum* as the out group species with bootstrap test (1000 replicates)

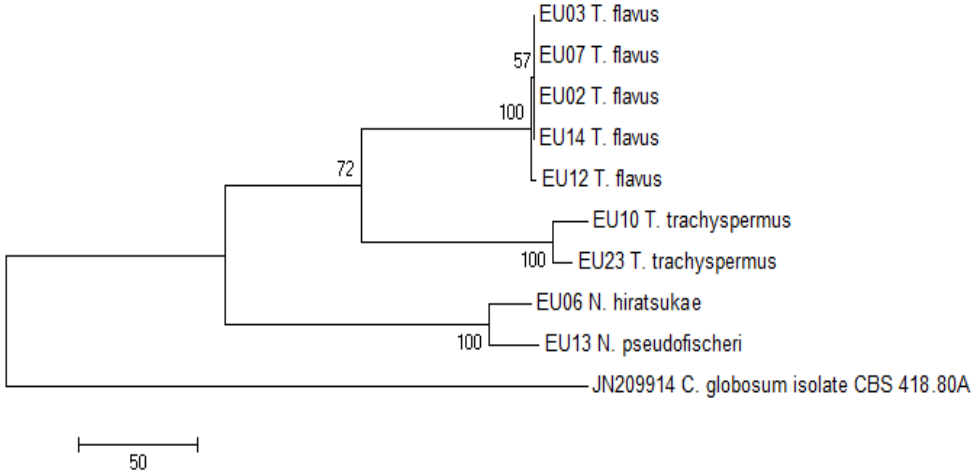

Fig. 4 Maximum parsimony tree of nine isolates in this study based on the three regions using *Chaetomium globosum* as the out group species with bootstrap test (1000 replicate

This study confirmed that five isolates (EU02, EU03, EU07, EU12 and EU14) are identified as *T. flavus* according to morphology and molecular taxonomy. There is yellow colony, ascomatal walls composed of loose hyphae, colony spreading broardly, ascoma intials consisting of vermiform (Domsch and Gams, 1993). The anamorph synonym of this species is *P. vermiculatum* which is the holotype of *P. dangeardii*. Both of these names were *T. flavus* which is the anamorph name (Samson *et al*., 2011). EU10 and EU23 are identified as *T. trachyspermus*. There is yellow colony, ascomatal walls composed of loose hyphae, ascomata white to creamish, ascospores 2~2.5 x 3~3.5 µm (Domsch and Gams, 1993). *P. spicucillium* is the anamorph of *T. trachyspermus* (Samson *et al*., 2011). One isolate (EU13) is identified as *N. pseudofischeri*. It shows white colony, ascospores are subglobose to broadly ellipsoidal with furrow and ridges. *N. pseudofischeri* anamorph synonym is *Aspergillus thermomutatus* which cause invasive fungal infections of human disease (Balajee *et al*., 2005). As a result, *N. hiratsukae* (EU06) is confirmed by molecular phylogeny but the activity of this isolate is not known. It must carefully work on this species because Guarro *et al.* (2002) reported for the first time that *N. hiratsukae*, an Ascomycetes which the asexual conidia resembles *A. fumigatus* causes a brain infection in a Brazilian woman. However, Hawksworth (2009) reported that *N. fumigata* is given to the sexual stage which is also asexually produced as *A. fumigatus* and the analogy of *A. nidulans* where its teleomorph *Emericella nidulans*.

Nine isolates belongs to Trichocomaceae were isolated from forest soils. These isolates were identified and confirmed species by morphology and three regions of ITS, *β*-tubulin and calmodulin. The morphology were shown two different groups of characters which identified as *Talaromyces* and *Neosartorya*. The nucleotide sequences were demonstrated and related to the four species: *Talaromyces flavus*, *T. trachyspermus*, *Neosartorya hiratsukae* and *N. pseudofischeri*. Some species of Trichocomaceae occur commonly and are important to both industry and medicine. Therefore, pathogenic fungi and their ability to secondary metabolite were also studied. However, a new classification system including both anamorph and teleomorph species will be investigating the relationship of some genera of Trichocomaceae.

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Gender identification of *Himantopus himantopus* using a PCR-based method

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The black-winged stilt (*Himantopus himantopus*: Recurvirostridae) has a wide distribution. In Thailand, there are permanent resident and migratory birds. The adult birds have very long pink legs and black wings. Crown and hindneck patterns vary from white to dusky-grey. In general, the gender can be identified by crown and hindneck color, white in female and black in male. However, in breeding season (April – June) of resident bird at Bueng Boraphet where the largest freshwater swamp in central Thailand, most of their crown and hindneck are white. So, this species are not clearly sexually dimorphic and the color of their crown and hindneck are not directly correlated with sex. In this work, a molecular approach was used to assess differences in coloration of feathers in relation to gender. DNA was amplified from the *chromo-helicase-DNA-binding* (*CHD*) genes that located on both Z (*CHD-Z*) and W (*CHD-W*) chromosomes. Using FTA®card and genomic DNA which extracted from a small volume of blood samples were used in this experiment. The resulting PCR products from 2550F/2718R primer showed fragments on a conventional agarose gel electrophoresis with size differences ranging from 100 bp between the two ZW alleles. Males were identified by the presence of a single band about 600 bp (*CHD-Z*) and females were identified by the presence of a second additional fragment length of approximately 500 bp (*CHD-W*). For the resident bird included 32 white and 2 black head, the ratio of female: male were 181 :6 which 2 black heads are male. On the other hand, the migratory bird included 32 white, 48 greyish and 12 black head, the ratio is 37 female and 46 male. Our results showed clear evidence for a sex- related decrease in the color on the heads of black-winged stilt.

Key words: *Himantopus himantopus* ,sex identification, *chromo-helicase-DNA binding* (*CHD*) gene

Introduction

The black-winged stilt (*Himantopus himantopus*: Recurvirostridae) has a wide distribution in nature. The adult birds have white body, needle-like

blackish bill, black wings and very long pinkish-red legs. According to gender, black-winged stilt can be identified by crown and hindneck pattern color. Male are typically all white and sometimes can have some variable grey or black while female are browner and may show grey and black (Lekagul and Round, 1991). Brumfield (2010) stated that this species is usually name based on its crown and hindneck patterns and colors. For example, the white-headed stilt/ pied stilt (*Himantopus leucocephalus*) which reside in Australia but also found in Borneo, Java and the Philippines.

In Thailand, black-winged stilt were divided into 2 groups (1) resident birds: non-migratory populations and (2) migratory (visitor) birds. There are listed as protected animal according to Wildlife Preservation and Protection Act B.E. 2535 (1992). The migratory birds migrate to Thailand during November and January. They mostly are found in swamp and wetlands where food resources are available such as Bung Boraphet; the largest freshwater swamp in central Thailand. The resident birds are also resided and breed in the wetland area. The breeding season of resident birds generally starts from April to June. However, the morphological of resident birds are mostly white crown and hindneck pattern from observation. Therefore, this species are not clear sexual dimorphism and the pattern color of crown and hindneck are not directly correlated with their gender. The gender are an important understanding behavior, social structure, breeding system, mechanisms and patterns of migration and estimating extinction risk.

The birds used traditional methods for sex identification (Cerit and Avanus, 2006; Morinha *et al*., 2012) such as sexually dimorphism, acoustic sexing, laparoscopy, cloacal examination, steroid sexing and cytogenetic analysis. These methods are slow, expensive and harmful in some cases. So, the molecular techniques for birds sexing were developed. Most species of birds can be identified based on *CHD* gene; *chromo-helicase-DNA-binding* located on sex chromosomes (Griffith *et al.,* 1998). Male birds are homogametic sex which has two Z sex chromosomes. On the other hand, female birds are heterogametic sex and have Z and W sex chromosomes that containing *CHD-Z* and *CHD-W*, respectively (Watson *et al.,* 2004). Nowadays, molecular technique is a more reliable method for identifying the sex of birds which are monomorphic. However, this technique has not been in black-winged stilt. Therefore, the main aims of this research are to assess differences in coloration of feathers in relation to gender of black-winged stilt. Including, examining genetics relationship of crown and hindneck colors and its species by using molecular technique.

Materials and methods

Sample collection

Thirty-four adult black-winged stilts were trapped by spring trapping method during breeding season (April-June) and ninety-two of migratory birds were trapped by cannon netting method during migration season (November-January) at Bueng Boraphet, Thailand. After being trapped, the bird measurements were made such as: wing, bill, head, tar, tail, weight and fat content. Secondly a small volume of blood sample was collected onto FTA®card (GE Healthcare, UK) by a puncture from the toe vein of the birds. Then photograph of the birds were taken especially their crown and hindneck pattern color. After all these processes the birds were released back to nature.

Purification of DNA onto FTA® Card

Blood samples in FTA®card were punched on dried blood sample (approximately 2 mm diameter). Place each disc in a PCR amplification tube and washed two times with 125 µl FTA Purification Reagent (GE Healthcare, UK). Each time the sample was mixed by pipetting up and down several times and incubates at 65°C for 10 min after which the liquid was removed. After that, 125 µl of 0.1 mM TE buffer was added and incubated at room temperature for 10 minutes. TE buffer was discarded and repeated the TE buffer step again. Finally the disk in PCR tube was dried at 65 ºC for 10 minutes.

PCR amplification and sequencing

For gender identification, the PCR reactions were used 3 primer sets, including P2/P8 primers (Griffiths *et al.,* 1998), 1237L/1272H primers)Kahn *et al.,* 1998 (and 2550F/2718R primers)Fridolfsson and Ellegren, 1999(. Amplification was performed in 25 µl total volume that contained 12.5 µl of 2X *Taq* Master mix (Vivantis), 1 µl of 20 µM from each primer set and 10.5 µl of nuclease free water. The conditions for PCR amplification conditions were a initial denaturing step at 95 ºC for 5 min, 35 cycles of 95 ºC for 45 sec, 50 ºC for 45 sec, and 72 ºC for 45 sec, and final extension at 72 ºC for 5 min. PCR products were electrophoresed through 1.5% agarose gel in 1X TBE buffer comparing with 50 base pairs DNA ladder (Vivantis) and stained with ethidium bromide. Then, PCR products were purified by GF-1 AmbiClean Kit (Gel &

PCR) from Vivantis. In addition, PCR products were sequenced by 1st BASE (Malaysia). Sequences were edited and analyzed by Bioedit and MEGA 6.

Results and discussion

Three primer sets (including P2/P8, 1237L/1272H and 2550F/2718R) were used for gender identification of *CHD* gene of black-winged stilt. The results were shown that P2/P8 primers are not specific for this bird and 1237L/1272H primers could not identify the gender (Fig.1). Nevertheless, 2550F/2718R primers were shown clearly different between male and female birds by fragments on a conventional agarose gel electrophoresis. The size differences ranged from 150 base pairs between the two ZW alleles. Fridolfsson and Ellegren (1999) show that the resulting PCR products from 2550F/2718R primers showed fragments on a conventional agarose gel electrophoresis with size differences ranging from 150 base pairs between *CHD-Z* and *CHD-W* allele. Male birds amplified a single band of approximately 650 base pairs. However, female birds amplified two bands 500 base pairs (*CHD-W*) and 650 base pairs (*CHD-Z*). Therefore, 2550F/2718R primers were used to identify the gender with all samples. Vucicevic *et al.* (2012) reported that the use of 2550F/2718R primers also gave good results in 50 species; most of them are in the same class as black-winged stilt. At the same time, P2/P8 primers were successful used for sexing in Eurasian Oystercatchers (*Haematopus ostralegus*) which is in Charadriiformes as same as black-winged stilt (Watson *et al.*, 2004). In these studies, the female chicken (*Gallus gallus domesticus*) was used as a positive control (Fig. 2: the last lane). However, some samples (7.1%) could not amplify (Fig. 2: lane 2-3). This failure may be from FTA®card purification step. In order to ensure success, FTA®card should be washed away all cell debris.

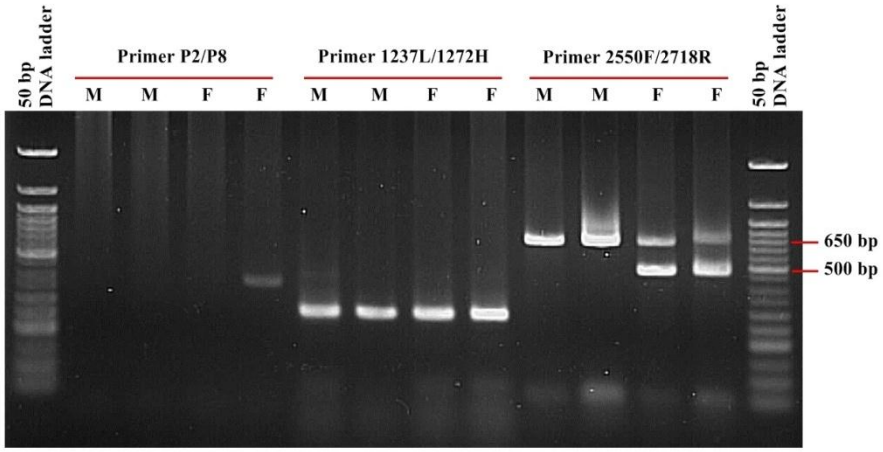

Fig.1 Gender identification of black-winged stilt using 3 primer sets were separated on 1.5% agarose gel. The first and last lane is 50 base pairs DNA ladder. Lane 2-5: samples were amplified by using P2/P8 primers. Lane 6-9: samples were amplified by using 1237L/1272H primers. Lane 10-12: samples were amplified by using 2550F/2718R primers

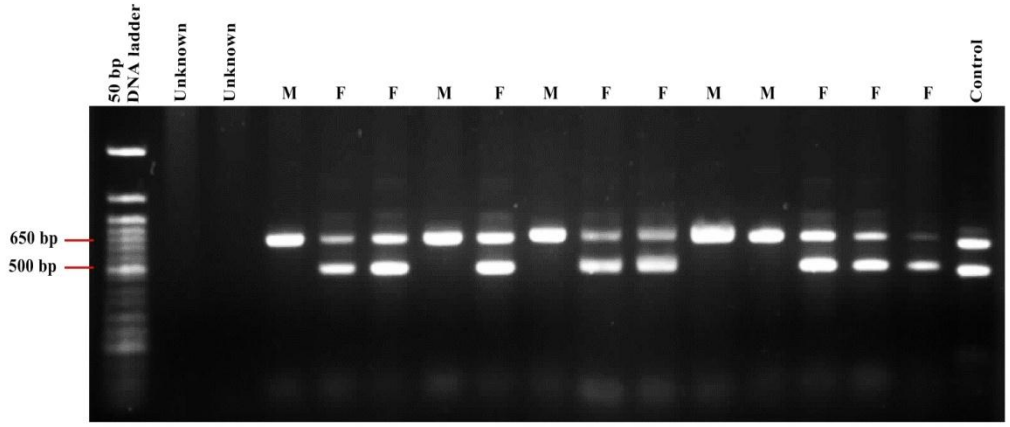

Fig. 2 DNA samples were extracted from FTA®card and were amplified by using 2550F/2718R primers. The first lane is 50 base pair DNA ladder, with black-winged stilt samples in lane 2 onwards. Samples with single bane are male and those with two bands are female. The last lane is female chicken

which shows two bands pattern as positive control

Table 1. Gender identification of black-winged stilts comparing with their crown/hindneck pattern colors during breeding and migratory season

Season	Crown/hindneck pattern color	Number of sample			Totals	
		Male	Female	Unknown		
Breeding	White	16	16	-	32	
	Black	2	-	-	2	
	Totals	18	16	-	-	34
Migratory	White	14	16	2	32	
	Black	5	6	1	12	
	Grey	22	15	6	43	
	Grey crown	5	-	-	5	
	Totals	46	37	9	-	92

The adult resident and migratory birds were separated into 2 and 4 patterns, respectively (Fig. 3). In breeding season of resident birds, there are white crown/hindneck and black hindneck pattern colors. However, there are (1) white crown/hindneck, (2) black hindneck, (3) grey crown/hindneck and (4) dusky-grey crown in migration season. From 126 samples, resident birds show that female: male were 181 :6 which 2 black hindneck are male. On the other hand, migratory birds were 37 female and 46 male (Table 1). Our results showed clear evidence for a sex- related decrease in the color on the heads of black-winged stilt.

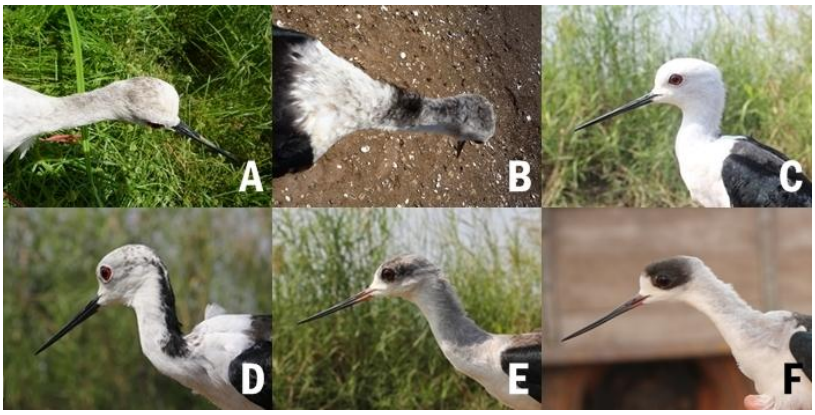

Fig. 3 The resident birds (A-B) and migratory birds (C-F) were separated by the crown and hindneck pattern color. A: white crown/hindneck, B: black hindneck, C: white crown/hindneck, D: black hindneck, E: grey

crown/hindneck and F: dusky-grey crown

In addition, PCR products from 2550F/2718R primers were analyzed by comparing *CHD-Z* allele from 9 resident and 12 migratory birds (Fig. 4). About 554 base pairs nucleotide sequence were shown 2 regions of single nucleotide polymorphism. The black column on left side presents a transversion mutation which involves exchange of purine (A) and pyrimidine (G). On the other hand, the black column on right side presents a transition mutation which involves bases of purine (A) and purine (T). This result corresponds to Faux *et al.* (2014) which stated that the conserved regions between *CHD-Z* and *CHD-W* of Southern Ocean seabirds contain some base changes.

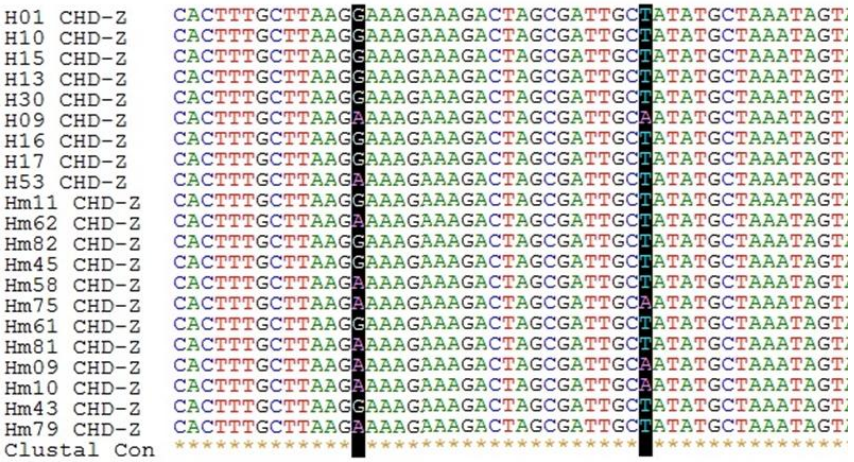

Fig. 4 Two regions of single nucleotide polymorphism on sequence of *CHD-Z* allele from 9 resident (H) and 12 migratory birds (Hm)

Conclusion

From 126 samples of black-winged stilt, the PCR of *CHD* gene could be detected 93% of samples for gender identification. Also, the use of FTA®card

is successfully to this study and less harmful to the birds. However, the results of the *CHD* were compared with the gender assigned to each bird by crown and hindneck pattern colors, it confirmed that the gender could not be identify by the colors alone. In the future, it would be better if DNA analysis and the biometrics of the birds to be used to compare. Additional, another gene and molecular techniques such as RAPD, SRAP and iPBS are interested for future research studies.

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Antagonism of *Chaetomium* spp and their ability to control citrus root rot caused by *Phytophthora parasitica* in Vietnam

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Phytophthora parasitica was isolated from root rot of citrus trees in the fields in Hung Yen and BacGiang provinces in Vietnam. It proved to be virulent isolate caused root rot of citrus seedling using root-dipped method. Many isolates of *Chaetomium* spp were isolated by baiting technique. It was morphological identified as *Chaetomium cupreum* (CC) and *Chaetomium globosum* (CG). The ED50 values of CG- methanol to inhibit *P. parasitica* was 16 ppm, and followed by CC-hexane 88 ppm, CC-ethyl acetate 97 ppm, CC-methanol 165 ppm, CG-hexane 185 ppm and CG -ethyl acetate 4487 ppm. It is proved the control mechanism as antibiosis. This is a part of doctoral research program and further investigations would formulate as biological fungicide and test in the fields which being in progress.

Key words: citrus root rot, *Chaetomium* spp, natural product

Medicinal herbal plants of Phetchaburi with anti-diabetic potential

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The mellitus is one of the common metabolic disorders acquiring around 2.8% of the world's population and is anticipated to cross 5.4% by the year 2025. Phetchaburi, the prevalence of diabetes mellitus is on increase and needs to be addressed appropriately. In this study area, herbal remedies are considered convenient for management of type 2 diabetes with postprandial hyperglycemia due to their traditional acceptability and availability, low costs and lesser side effects. Ancient times, plants have been an exemplary source of medicine. Ayurveda and other

Thai literature mention the use of plants in treatment of various human ailments. Thai has about 57 plant species and among them, several thousands have been claimed to possess medicinal properties. Research conducted in last few decades on plants mentioned in ancient literature or used traditionally for diabetes have shown anti-diabetic property. The present paper reviews 12 such plants that have been used in the Thai medicinal herbs and have shown experimental or clinical anti-diabetic activity. Thai plants which are most effective and the most commonly studied in relation to diabetes and their complications are: Aegle marmelos(L.)Corr.:Matoom (Thai), Allium sativum Linn.:Gatium (Thai), Capsicum acuminatum Fingerh.:Prik Chee Fah(Thai), Coccinia grandis (L.) :Tumluang(Thai), Lagerstroemia speciosa L. Pers.:Intaninnum(Thai), Leucaena glauca Benth.:Glatin(Thai), Momordica charantia :Marakeenok(Thai), Nelumbo nucifera :Bua(Thai), Ocimum sanctum :Gapao(Thai), Phyllanthus amarus : Luktaibai(Thai), Senna siamea Lam :Keelek (Thai) and Tinospora cordifolia :Boalapetch(Thai). All plants have shown varying degree of hypoglycemic, anti-hyperglycemic activity. The review describes some new bioactive drugs and isolated compounds from plants Thus, from the review majorly, the antidiabetic activity of medicinal plants is attributed to the presence of memosine, tolbutamide, capsaicin, charatin, and other constituents which show reduction in blood glucose levels. The review also discusses the management aspect of diabetes mellitus using these plants and their active principles.

Key words : Thai plants; Ayurveda; Diabetes mellitus; Plants; Herbal medicine

Slide assay of inhibiting properties of nanochitosan against *Sclerotioum rolfsii*

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This study assessed slide assay as an innovative adaptation protocol in determining inhibitory properties of extracts. It also evaluated the inhibitory properties of chitosan, nanochitosan and superchitosan, against *Sclerotium rolfsii.* Slides inside petri plates were sterilized covered with potato dextrose agar medium. They were inoculated with sclerotia in the middle part, and the test chitosan on one side and distilled water as control on the other side. The growth of the fungus from middle of the slide towards the test extract and control were monitored for three days. Percent growth of inhibition were computed. Visual evaluation of the results showed inhibitory properties of nanochitosan and superchitosan against *S. rolfsii.* Significantly less growth were observed in the *Sclerotium sp*. in the control side of the slide compared the one with extracts. Significant higher percent of inhibition was observed on the third. Further analysis implied that superchitosan and nanochitosan had comparable inhibitory capability against *Sclerotium sp.* Results of this protocol evaluation imply that slide assay may be used to assess antifungal properties in shorter period of time with more economical use of media.

Key words: slide assay, antifungal assay, nanochitosan, *Sclerotium sp*.

Nutraceutical attributes of the three strains of *Pleurotus ostreatus*

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This study was conducted to analyze the presence of some important mycochemicals and nutritional compositions, and evaluate the antibacterial and antioxidant activity of fruiting bodies of the three strains of *Pleurotus ostreatus* namely; Indonesia, USA and Spain grown on rice straw-sawdust (7:3) based substrate formulation. The mycochemical screening was done using the qualitative test tube methods to detect the presence of flavonoids, phlobatannins, cardiac glycosides, tannins, saponins and alkaloids. Crude protein, crude fat, crude fiber, ash, moisture content, total carbohydrates and energy value were also analyzed. The antibacterial activity of ethanol and hot water extracts of *P. ostreatus* against *Escherichia coli* and *Staphylococcus aureus* was evaluated. DPPH radical scavenging assay and Folin - Ciocalteu Assay were used to assess the antioxidant activity of the mushroom samples. Mycochemical screening showed that saponins, alkaloids and tannins were detected in both Indonesia and US strains while cardiac glycosides and alkaloids were found present in Spain strain. Flavonoids and phlobatannins were not detected in all the *P. ostreatus* strains. Proximate analysis showed that Indonesia strain had higher amounts of ash (5.31%), crude fiber (7.79%) and crude fat (2.48%) while USA strain had higher amounts of moisture (11.96%), crude protein (14.61%) and carbohydrate (59.82%). However, Spain strain showed the highest amount of energetic value (316.94 kcal). Antibacterial assay of the hot water and ethanol extracts of *P. ostreatus* strains showed negative effects against *E. coli* and *S. aureus*. Indonesian strain significantly registered the highest scavenging activity (29.22%) and phenolic content (384.51 mg AAE/g sample) while US had lowest scavenging activity (25.92%) and phenolic content (257.57 mg AAE/g sample).

Key words: nutraceutical, strain, Pleurotus

Potential use of collagen extracted from goat *(Capra hircus)* skin infused with clove essential oil (*Syzygium aromaticum*) as an active packaging

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A study which focused on the potential ability of collagen extracted from goat skin and infused with varying concentrations of clove essential oil as an active packaging was conducted. Collagen was extracted from the goat skins through alkaline and acidic extractions and was dehydrated into flake form. The resulting flakes were then formulated as the film forming solution (FFS). Clove essential oil (CEO) in different concentrations (0.5%, 1.0%, and 1.5%) were infused to the FFS and were subjected to various analyses which were all made with three replications. Results showed that the gelatin forms a stable state at 6% and turned into a faintly opaque and elastic film upon drying for 24 hours. The antibacterial property of the film solutions was tested against *Staphylococcus aureus* and showed negative results on all concentrations. The antifungal effect of the films against *Aspergillus sp.* was also tested and showed inhibitory effects. Treatments 2 and 3 showed ++, weak inhibitions revealing a significant difference from treatment 1 on all test runs. DPPH Radical scavenging test revealed that the films are capable of protecting the food system from oxidation. The results on the light permeability test suggested that upon the addition of the extract, regardless of the assigned concentrations, the penetration of UV light has been significantly decreased compared to that of a film without the extract. The films were also subjected to a water vapor permeability test and showed that the films were significantly capable of retarding moisture migration into and from the system. Based on the results, FFS from goat skin collagen has a potential to be an effective active packaging.

Key words: goat skin collagen; clove essential oil; active packaging

SESSION 5 : Production Technologies

The comparison on growth, yield, cost and benefit of 4 species of vegetables grown in deep and shallow water hydroponics with different plant spacing

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This study aimed to compare growth, yield, cost and benefit of the 4 species of vegetables namely red coral, green lettuce, green cos, and Chinese cabbage grown in deep water hydroponics with plant spacing of 20 cm x 25 cm (DWH20 x 25), deep water hydroponics with plant spacing of 10 cm x 12 cm (DWH10 x 12), and shallow water hydroponics with plant spacing of 20 cm x 25 cm (SWH20 x 25). The results showed that DWH20 x 25 and SWH20 x 25 had a tendency to give higher number of leaves per plant than DWH10 x 12. The plants grown in DWH20 x 25 and DWH10 x 12 were taller than in SWH20 x 25. Shoot dry weight per plant and total dry weight per plant in DWH20 x 25 and SWH20 x 25 were similar, and both were higher than that in DWH10 x 12. However, DWH10 x 12 yielded higher than in both DWH20 x 25 and SWH20 x 25 by about 56.30% and 45.70%, respectively. There was an interaction on plant density and yield between vegetable species and hydroponics type with plant spacing, as the plant density and yield of Chinese cabbage were the highest in DWH10 x 12 compared to the rest, while the yield of red coral was the lowest in DWH20 x 25. Chinese cabbage vegetable production in DWH10 x 12 was the most feasible for investment, indicated by the highest value of benefit and cost ratio of 2.28.

Key words: hydroponics system, plant spacing, yield, Chinese cabbage, green cos, cost, benefit

Effects of number of seedlings on growth, yield, cost and benefit of 2 rice genotypes in transplanted fields

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This experiment was conducted during the dry season of December, 2010 until May, 2011, in the farmer's irrigated rice fields at Suan Luang village, Pongpawaey Subdistrict, District of Den Chai, Phrae province in Thailand to assess plant growth, yield, cost and benefit of RD14 and San-pah-tawng1 rice genotypes grown in transplanted fields under crop establishment methods using 1, 2, 3, and 4 seedling(s)/hill. Plant height of RD14 rice genoype was significantly taller than San-pah-tawng1 rice genotype. Grain yield of RD14 rice genotype was significantly higher than San-pah-tawng1 rice genotype; mainly due to RD14 rice genotype having had higher filled grain number per panicle and harvest index. Grain yield was, however, influenced by crop establishment method and rice genotype as RD14 rice genotype grown under crop establishment method using 1 seedling/hill yielded significantly higher than the controlled treatment (crop establishment method of RD14 rice genotype using 4 seedlings/hill). The two rice genotypes grown under crop establishment methods using 4 seedlings per hill had higher costs of production than other crop establishment methods using lesser number of seedlings per hill, mainly due to the higher use of seed rate per hectare for seedling preparation that raised the cost of production. The seeds' cost for seedling preparation for RD14 rice genotype under crop establishment methods using 1 seedling/hill could be saved by about 75% compared to that crop establishment method using 4 seedlings per hill. The net profit derived from RD14 rice genotype grown under crop establishment methods using 1 seedling/hill was considerably higher by 46% than of the controlled treatment. Therefore, the crop establishment method using 1 seedling/hill for RD14 rice genotype was the most feasible for investment in transplanted rice production indicated by the highest value of benefit-cost ratio (B/C ratio is 1.17) compared to the rest of the treatments.

Key words: RD14, San-Pah-Tawng1, yield, harvest index, benefit-cost ratio

Product development of sweet fermented rice products of germinated back native rice

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Product development of sweet fermented rice products of germinated back native rice was determined. Experimental design by using Randomized Complete Block Design (RCBD) with composed of 6 treatments. There are the ratio of glutinous rice and germinated back native rice 100:80 0:60 20:40 40:20 60: 80and 0:100, respectively. Sensory evaluation was determined by using 9-point hedonic scale. The results was found that most of consumer liked treatment 2 (the ratio of glutinous rice and germinated back native rice; 80:20) with the highest score 8.10 (like)

and significantly different from another treatments ($p \leq 0.05$). Then pH, total soluble solid, % alcohol and anthocyanin content were studied. The results exhibited that the similar trends was found in all of treatments. The physical and chemical and microbiological properties of final product are L* a* b* (12.44,11.81 , 28.72), chemical composition are moister, ash, lipid, protein, fiber and carbohydrate content 1.59 3.11 0.35 0.29 55.86and 38.80 %, respectively, total soluble solid (5.23 % brix), pH (4.34), alcohol content (16.67 %(, the total microorganism (8.00 $x10^3$ CFU/gram) and yeast and molds (5.30 $x10^3$ CFU/gram) .Finally, the free radical scavenging capacity were assay by DPPH method shown that SC_{50} value were significantly higher than control (59.70 ± 1.28 and 199.38 ± 1.28 2.59 mg/ml, respectively) ($p \leq 0.05$). In addition, gamma-aminobutyric acid (GABA) content were found 6.53 mg/100 g. In conclusion, the sweet fermented rice products of germinated back native rice from this research could be a new healthy product in the future.

Key words: sweet fermented rice, germinated back native rice, radical scavenging capacity, gamma-aminobutyric acid

Callus induction and growing cell suspension culture of jow haw rice (*Oryza sativa* L.)

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Mature embryos of rice (*Oryza sativa* L.) varieties Jow Haw was cultured on solid MS (Murashige and Skoog, 1962) and NB (Nitsch and Nitsch, 1969) medium supplemented with 0.5, 1, 2, 3 and 5 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D), 30 g/l sucrose, 1 g/l proline, 100 mg/l casein hydrolysate and 2.6 g/l phytagel. An optimum concentration of 3 mg/l 2,4-D in NB medium was found to be effective for callus induction which induced the biggest size of calli. Transferred the calli to liquid NB medium containing 3 mg/l 2,4-D, 30 g/l sucrose, 1 g/l proline and 100 mg/l casein hydrolysate. Study the growth phases at 0, 3, 6, 9, 12, 15, 18 and 21 days respectively, were determined by measuring fresh and dry weight of the cell. And viability of cell suspensions were determined by the method of fluorescein diacetate. The living cell of cell suspensions showed green fluorescence.

Key words: Callus induction, Cell suspension, Growth curve, Jow Haw, RD6, Living cell

Introduction

Rice is the most important human food, eaten by more than half of the world's population every day. There are two types; glutinous and non-glutinous rice. In Asia where its covers half of the arable land used for agriculture in many countries (Cantrell and Hettel, 2004). Nevertheless, rice yield and quality are affected by pests and diseases, as well as by environmental stress. The quantity of rice has decreased which insufficient for consumption. Therefore, micropropagation of rice is considered as an important technique to produce sufficient food supplies. A success in micropropagation depends on the genotype of plant, type of the explants, composition and concentration of the basal salt and organic components and plant growth regulators in the culture medium (Ge *et al*., 2006). Amount of studies on increase efficiency of callus induction and cell suspension cultures have been reported in many rice cultivars. However, there is no report on the non-glutinous rice cultivar Jow

Haw. Jow Haw is an upland rice and traditional rice found at the northern of Thailand. This grains shall possess the characteristics and size as follows: general characteristic is long grain, average length and width of the fell grain rice without breakage are not less than 7.0 mm. and 3.0 mm. Then, can resistance to blight diseases. When cooked, the grains' texture becomes tender. Therefore, the objective of this study was to found the suitable medium and concentration of plant growth regulators (2,4-D) for maximum callus induction and the biggest size of calli, growth curve of cell suspension cultures and viability for living cell from mature seeds of the rice cultivar Jow Haw.

Materials and methods

Surface sterilization

Jow Haw seeds were dehusked and surface sterilised in 70% ethanol for 1 min and then shaking in 20% of sodium hypochloride 30 min. After rinsing 3 times with sterile distilled water. Seeds were kept on sterilized filter paper in a Petri dish to remove excess water prior to transfer onto culture media.

Callus induction

The sterilised seeds were cultured on solid MS and NB media contained 0.5, 1, 2, 3 and 5 mg/l 2,4-D, 30 g/l sucrose, 1 g/l L-proline, 100 mg/l casein hydrolysate and 2.6 g/l phytagel. The pH of all media were adjusted to 5.8 before autoclaving at 121 ºC for 15 min. The cultures were maintained at 25±1°C under dark condition for 4 weeks. Each treatment consisted of 60 seeds.

The frequency of callus induction was counted after 4 weeks and calculated according to the following formula:

$$\text{Callus induction frequency (\%)} = \frac{\text{No.of seeds produced calli x 100}}{\text{No.of seeds cultured}}$$

And measure the size of callus by vernier calipers and calculated according to the following formula:

$$\text{Mean size of callus formation (mm}^3\text{)} = \frac{\Sigma\ (\text{width x length x height) of calli}}{\text{No.of seeds that produced calli}}$$

Establishment of cell suspension cultures

Cell suspension cultures of Jow Haw rice was initiated from callus, 0.15 g of callus were transferred into tissue culture bottle 4 oz. containing 10 ml NB or MS liquid medium supplemented with 0.5, 1, 2, 3 and 5 mg/l 2,4-D that induced the biggest size of callus and combined 30 g/l sucrose, 1 g/l L-proline, 100 mg/l casein hydrolysate. The pH of all media were adjusted to 5.8 before autoclaved. The cultures were incubated on a rotary shaker at 120 rpm and maintained at 25±1 ºC.

Growth Measurements of cell suspension and cell viability

Cell suspension growth was study the growth phases at 0, 3, 6, 9, 12, 15, 18 and 21 days, respectively. Stable suspension cultures were used to demine the growth curve. The growth of the cell suspension cultures were determined by measuring fresh and dry weight of the cell every three days. Three replications were used for each experiment. The fresh weight of suspension cultures was measured by removing the medium. Cells were separated by filtration and washed with distilled water under vacuum. Dry weights of the fresh cells were determined after drying at 110 °C for 60 min, in a hot air oven until constant weight (Poeiam and Saengdeuan, 2000).

Study the viability of the cell suspensions with a fluorescent microscope. Suspension cells were transferred onto a microslide and stained with 0.5% fluorescein diacetate (FDA), covered with a cover slip. Under the fluorescent microscope, the living cell of cell suspensions showed green fluorescence.

Table 1. Callus induction frequency (%) and mean size of callus (mm^3) of Jow Haw rice which were cultured on solid MS and NB media supplemented with 0.5, 1, 2, 3, 5mg/l 2,4-D, 30g/l sucrose, 1g/l L-proline, 100mg/l casein hydrolysate and 2.6 g/l phytagel after 4 weeks.

Media	No. of seeds cultured	Concentration of 2,4-D (mg/l)	Frequency of callus induction (%)	Mean size of callus formation (mm^3)
MS	60	0.5	26.67	90.45
	60	1	45.00	84.64
	60	2	30.00	121.84
	60	3	38.33	79.39
	60	5	35.00	57.65
	60	0.5	27.00	112.02
	60	1	33.00	120.75

NB	60	2	25.00	135.99
	60	3	38.00	172.50
	60	5	35.00	114.81

Results and discussion

In present study the efficiency of MS and NB media containing different concentrations of 2,4-D was tested for callus induction in Jow Haw rice. MS and NB media are two well know media used to induce callus and regenerate plants in rice genotypes. The callus was induced successfully in all media and formed mostly embryogenic calli, which creamy, dry and compact appearance (Figure 1).

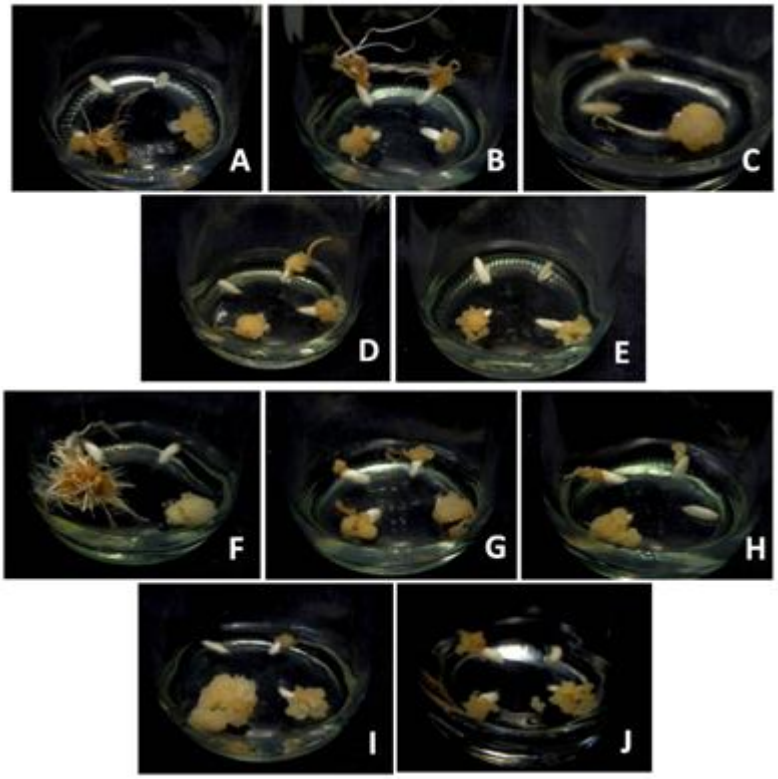

Fig. 1 Callus from seeds of Jow Haw rice cultured on solid MS (A-E) and NB (F-J) media supplemented with 30 g/l sucrose, 1 g/l L-proline, 100 mg/l casein hydrolysate, 2.6 g/l phytagel and concentration of 2,4-D; (A, F) 0.5 mg/l 2,4-D, (B, G) 1 mg/l 2,4-D, (C, H) 2 mg/l 2,4-D, (D, I) 3 mg/l 2,4-D and (E, J) 5 mg/l 2,4-D after 4 weeks in culture.

The response of the seeds to different media and concentrations of 2,4-D for callus induction, the percentage of seeds producing calli were calculated for all media. That found the percentage of frequency of callus induction were not much difference between MS and NB medium. However, the formation of callus was observed, suggesting that 2,4-D play a crucial role in callus induction of rice as described by Chen *et al*. (1974), Maeda (1980) and Bajaj (1991).

Callus was initiated from seeds after two weeks of cultivation. The proliferation of callus was continued until the fourth weeks, Then the frequency of callus induction and size of callus were determined. The results are show in Table 1. The biggest size of callus was 172.50 mm^3 on NB medium containing 3 mg/l 2,4-D, followed by 2 mg/l (135.99 mm^3), 1 mg/l (120.75 mm^3), 5 mg/l (114.81 mm^3) and lowest 0.5 mg/l 2,4-D (112.02 mm^3). The 0.5 mg/l 2,4-D supplement developed shoot and root more than other 2,4-D concentrations as seen in Carsomo and Yoshida (2006). Mean size of callus formation, which cultured on NB medium was found bigger than MS medium. The difference in the composition of culture medium can result in variation in callus induction (Torbet *et al.,* 1998). NB medium composed of nitrogen sources as N_6 medium and PP medium (Poonsapaya *et al.,* 1989) as recommended by Rueb *et al.* (1994). It is, perhaps due to the reason that NB medium contained more nitrogen than the MS medium. However, Raina (1989) reported that 2,4-D is the most suitable auxin for callus induction of rice in tissue culture, although the optimum concentration of 2,4-D varied depending on the explants source and genotype. Results of the present study were in agreement with those of Visarada *et al*. (2002) cultured four varieties of rice; Seshu, Nagarjuna, Rasi, and Jaya for callus induction on NB medium containing 3 mg/l 2,4-D found that percentage of callus induction were highest when compared to other concentrations of 2,4-D (57.1, 81.4, 94.1 and 78.1 %, respectively). Tariq *et al*. (2008) reported Fakhre Malakand rice gave highest mean weight (0.26 g) on N_6 medium containing 3 mg/l 2,4-D. In case of Basmati 370, the callus induction frequency was increased by increasing the concentration of 2,4-D from 1 to 2 mg/l and was maximum at 3 mg/l (Rsahid *et al*., 2003)

Table 2: The measurement of fresh and dry weight for cell suspension cultures of Jow Haw rice in liquid NB medium supplemented with 3 mg/l 2,4-D, 1 g/l L-proline, 100 mg/l casein hydrolysate and 30 g/l sucrose for 21 days.

Days	Fresh weight (g/10 ml)	Dry weight (g/10 ml)
0	0.1767	0.0059
3	0.2923	0.0066
6	0.2956	0.0083
9	0.2961	0.0117
12	0.3345	0.0169
15	0.3407	0.0200
18	0.2889	0.0122
21	0.2881	0.0095

Calli cultured ineitiated from embryogenic callus was cultured in liquid NB medium supplement with 3 mg/l 2,4-D. Growth rate was measured by fresh and dry weight at 3 days intervals, an exponential growth phase of cell suspension within the lag phase in the range of 0-3 days, 6-12 days during the log phase, stationary phase during the period of 12-15 days, and the period of the death phase after 15 days, which were presented in Figure 2(A-B). After 15 days, calli were decreasing and browning Bushra *et al.* (2009) reported that the browning subsequent death of explants and cultures could be attributed to the oxidation of polyphenols. The cell suspension in the log phase, that fast growth. Therefor, suitable to be used in activities such as transfer into fresh medium or used for regeneration. In this reported of Lima *et al*. (2008) reported that growth deceleration occurs as a result of the usage of nutrients and the accumulation of toxic substances in the culture medium. It is appropriate to subculture the callus at the beginning of this phase. Which found the maximum growth rate fresh and dry weight cell in the suspensions culture was increase rapidly approximately 12-15 days of culture. On 15th, the maximum of fresh weight and dry weight were 0.3407 and 0.0200 g per 10 ml of media, respectively (Table 2). The suspension cultured embryogenic calli showed form, compact, yellowish, nodular, easy dispersion and fast growth of cells in liquid medium in Figure 3A and Figure3B showed cell suspension scanning from the brightfield microscope for 21 days. Then, study the characteristics of cell suspension under microscope fluorescence at wavelength 440-480 nm. The live cell and dead cell in medium were observed the living cells of cell suspensions showed green fluorescence but death cells were not stain after staining 0.5% fluorescein diacetate (FDA) in figure 3C.

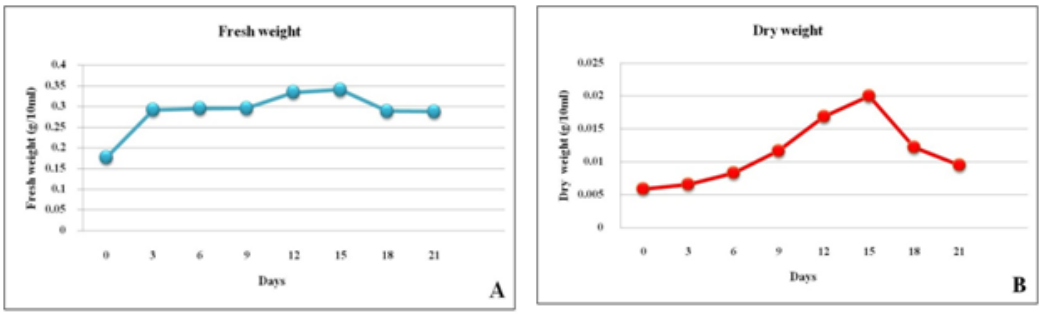

Fig. 2 Growth rate of fresh (A) and dry (B) weight cell for suspension of Jow Haw rice in liquid NB medium contained with 3 mg/l 2,4-D, 1 g/l L-proline, 100 mg/l casein hydrolysate and 30 g/l sucrose for 21 days.

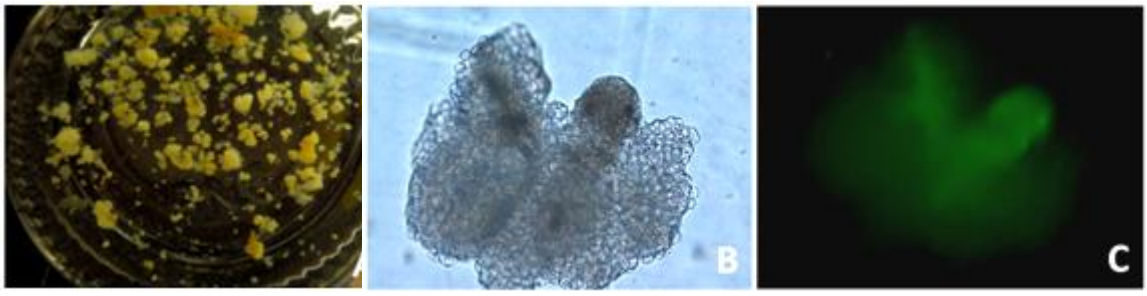

Fig. 3 (A) Cell suspension cultured on liquid NB media supplemented with 3 mg/l 2,4-D, 1 g/l L-proline, 100 mg/l casein hydrolysate and 30 g/l sucrose. (B) Cell suspension scanning from the brightfield microscope for 21 days. (C) Viability of suspension cells was determined by fluorescein diacetate.

Conclusion

The current study indicates that the NB medium supplemented with 3 mg/l 2, 4-D gave the biggest size callus (172.50 mm^3) from matured seeds of Jow Haw rice**.** Cell suspension cultures in 10 ml liquid NB medium supplemented with 2,4-D concentration of 2 mg/l, were found the highest of fresh weight and dry weight were 0.3407 and 0.0200 g/10 ml of media, respectively. The maximum growth rate is after 12-15 days of culture. The

living cell of cell suspension showed green fluorescence. The method presented here will be useful in biotechnological approaches to improve Jow Haw rice (*Oryza sativa* L.) through in vitro induced artificial mutations using radiation such as gamma-rays, for improving this world's staple food crop either to increase yield or to improve nutritional quality.

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Development and stabilization of vacuum-packed chevon caldereta wrapped in banana leaves (*Binalot*)

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This study purported to determine the appropriate packaging materials for vacuum-packed, thermally processed chevon calderetta with rice wrapped in banana leaves (binalot). Formulation of recipe for chevon calderetta that minimizes or eliminates the "goaty" smell and establishment of correct serving size and proportion of calderetta and rice were initially performed. Calderetta with rice was wrapped in banana leaves and vacuumed packed using two retort pouches (Stand-up pouches (SUP) Laminate and HYPAC Pouch) having four layer configurations. Pouches filled with 125 g of caldereta and 125 g of rice were heat-treated to 240 0F for 70 minutes in a water retort with overriding pressure. These two packaging materials were tested in chevon caldereta "binalot" and subjected to heat penetration study, commercial sterility, chemical analysis and cursory sensory evaluation. Retort cooked chevon caldereta "binalot" are heat-treated to a lethality (F0) of 6.0 minutes as this would guarantee consumer safety and low levels of economical spoilage. Results showed that the pH and water activity significantly differ prior processing and after heat treatment. However, pH and Aw of the retort-cooked product vacuum-packed in two pouches did not change much and did not differ significantly ($P>0.05$) after processing. Both products are acceptable after cursory inspection of sensory characteristics. Moreover, HYPAC pouch packaging did not withstand the thermal process and showed signs of leakage while the laminated SUP on the other hand, maintained its hermetic seal. Based on commercial sterility test, results revealed that product received sufficient thermal processing condition to achieve commercial sterility of the product, thus, indicating negative growth of microorganisms using both packaging materials. Hence, SUP Laminate was the most appropriate packaging material for vacuum-packed chevon caldereta "binalot".

Key words: chevon; caldereta; heat penetration; commercial sterility; retort pouch; binalot

Effect of substituting urea – wood ash treated corncobs for maize on chemical composition, rumen degradability and *in vitro* gas production parameters

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An experiment was conducted to determine the effect of substituting urea – wood ash treated corn cobs for maize on chemical composition, rumen degradability and in vitro gas production parameters. The urea – wood ash treatments were: T1 (100% urea), T2 (75% urea + 25% wood ash), T3 (50% urea + 50% wood ash), T4 (25% urea + 75% wood ash), T5 (100% wood ash). The result for the chemical composition showed that there were no significant differences in the neutral detergent (NDF) fibre and acid detergent fibre (ADF) of all the treatments except for the control (T6) with 100% maize which has lower NDF and ADF. The result for the rumen degradability parameters showed that there were no significant differences (P>0.05) in all the parameters measured – a, b, c, a + b and ED. Result for the in vitro gas production profile showed that a values for T6 and T5 did not differ statistically (P>0.05), also treatments 1, 2, 3 4 and 5 were statistically the same. The same trend was observed for the potential gas production (a + b) fractions. The result also showed that the insoluble gas production (b) fractions were statistically the same (P>0.05) for all the treatments. It was concluded that 100% wood ash treated corn cobs could effectively replace maize grain in ruminant diets.

Key words: Wood ash, Rumen degradability, In vitro gas production.

Practices in extension services: Basis for the formulation of manual of operation

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This research assessed the practices in extension services as a basis for the formulation of a manual of operation for extension of Quirino State University from June 2014 – October 2014. It investigated and described: the profile of the respondents according to sex, age, civil status, ethnic affiliation, highest educational attainment and years of experience in extension services. It determined the existence of significant difference on the frequency of usage of training

modalities and extension services, extent of benefits derived in extension activities, degree of seriousness of problems encountered along the different extension services. The study used the descriptive research design. Questionnaire was used to gather information from 59 extension coordinators and extension workers. Data were analysed using frequency counts and percentages, means, t-test, analysis of variance and scheffe' test were utilized. Extension workers belong to the age range 21- 30 mostly females and married. Most of the respondents are Ilocano, Masters Graduate and 1-5 years of experience in extension activities. Results revealed that individual method along electronic media/computer described as often, group method along field days is described as often, mass media along motion pictures is described as always. Environmental education and management on waste management described as often, alternative learning system on capability building program is described as often, nutrition, health and family welfare on information drive on disaster management is described as often, socio-economic activities on food processing is described as often, information technology on manipulation of computer and spread sheet is described as rarely, and community welfare on first aid is described as often. Office calls on gender, lecture on civil status, meetings on age, radio on ethnic affiliation and highest educational attainment, project visuals and lantern slides on age and ethnic affiliation, graphic and display formats on ethnic affiliation, and motion pictures on ethnic affiliation is significant . Directing on highest educational attainment, awareness of cultural values also on highest educational attainment is significant. Lack of community involvement on ethnic affiliation, lack of administrative support on civil status, peace and order on age is also significant. Gender, civil status, ethnic affiliation and highest educational attainment affect the extension modalities in extension activities. Age and civil status also affects the extension services in extension activities. Highest educational attainment affects the benefits derived in extension activities. Ethnic affiliation, civil status, and age also affect the degree of seriousness of problems encountered in joining the activities. Findings suggest that use of extension modalities and extension services should be strengthened, anticipation on desired outcomes should be considered, extension services should be sustained, expansion of extension activities should be done, proper planning is highly recommended, capability of extension services should be maintained, linkages should also be retained, Adopt-a-Community and School Program should still be sustained and economic and social impacts should be improved.

Key words: extension, services, operation

The efficacy of insecticidal control in pomelo fruit fly IPM program in Nakhon Si Thammarat Province, Thailand

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The major problem of pomelo production is fruit fly **(**Tephritidae; Diptera),whose damage causes fruits to fall from the trees so that it is necessary for farmers to use chemicals for controlling continuously. Six methods were used on IPM Program for controlling fruit flies in Taptim Siam Pomelo orchards in Pakpanang district, Nakhon Si Thammarat province, Thailand, from May 2012 to August 2013, with 4-time spraying, 10 days apart during the period of 3-month-old pomelo fruits. The experimental design using RCBD with 3 replications and 6 methods (M) was: 1.(M1)imidacloprid%10 SL+imidacloprid%10 SL+fipronil 5%SC+ imidacloprid%10 SL, 2.(M2) abamectin 1.8% EC+ imidacloprid%10 SL+ fipronil 5%SC+ abamectin 1.8% EC, 3. (M3) petroleum oil 83.9% EC + imidacloprid%10 SL+ fipronil 5%SC+ petroleum oil 83.9% EC, 4.(M4) Thai neem extract (aza. 0.05%)+ imidacloprid%10 SL+ fipronil 5%SC+ Thai neem extract (aza. 0.05%), 5.(M5: farmer method) abamectin 1.8% EC+ abamectin 1.8% EC + abamectin 1.8% EC +abamectin 1.8% EC and 6.(M6) control (non treated). The results revealed that, at the fruit harvesting period (6 months), the highest effectiveness method was M5 (81.35%), followed by M1, M2 and M3 at 81.29 81.29 and 78.22 %, respectively. The lowest effectiveness method was M4 (76.98%) compared with control (non treated).

Key words : Taptim Siam Pomelo, imidacloprid , fipronil , petroleum oil ,Thai neem, abamectin.

SESSION 6 : Rural Development, Socio-Economics and Social Science

Going organic: Understanding the organic vegetables production environment in Central Luzon, Philippines

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Organic vegetable production is a system based on the principle of taking care of nature accounting all life forms. It is a progressing industry in the Philippines given the increasing need for healthy and safe food and in effort to contribute in protecting the environment. The research aimed to characterize organic vegetables production environment in Central Luzon, Philippines. Survey, key informants interview and focus group discussion were used in generating data from 72 organic vegetable farmers and 32 conventional vegetable growers from the provinces of Nueva Ecija, Pampanga, and Zambales. Descriptive statistics, cost and return, input utilization, technology attributes, and extent of technology utilization were used in data analysis. Results indicate that despite the noted inadequacies, the bio-physical, socio-economic and institutional environment of organic vegetable production in Central Luzon can provide a good opportunity that can be tapped in the promotion and adoption of organic vegetable production in the region. he major organic vegetables raised across sites were eggplant, tomato, ampalaya and stringbeans. The common organic vegetable production technologies being adopted were the use of organic fertilizers, use of bio-pesticides, crop rotation, compost application, green manuring, use of biological control and mulching. As to farm management practices, the vegetable growers generally relied on their long years of experience in vegetable farming, infusing knowledge learned from the trainings in the use of organic inputs particularly in land preparation, nutrient management and control of pest and diseases. The returns in organic vegetable production in all sites is promising given the acceptable, at par and even better yield per 1000 sq m, net income, and return to total operating expenses compared to conventional vegetable production. There is generally very few organic farmers in Central Luzon at the time of survey with evident low utilization of organic vegetable production technologies. While they have a good understanding of the concept of organic farming, the capacity of the farmers to adopt organic farming standards including labelling and certification is generally low. The expanding vegetable organic vegetable industry in the country, the potential area for expansion, potential market and value adding activities, GOs and NGOs support and the evolving legislation on organic agriculture were the cited opportunities that can be taken into advantage in an effort to push for a vigorous organic vegetable production in the region. The risk, problems and constraints in organic vegetable production are many, but can be addressed through an integrated organic vegetable production program in the region. This calls for a holistic organic consciousness campaign,

prioritization and localization of organic vegetable production, intensifying capability building, subsidizing and localizing organic certification, and effective price monitoring and dissemination mechanism.

Key words: organic vegetables, organic technologies, attributes, utilization

Assessment of veterinary needs in municipalities of the third district of Cagayan: A benchmark survey for extension

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The study was generally conducted to generate information in extension services particular in Veterinary Medicine practices at the seven municipalities of District III of Cagayan. Specifically, the research aimed to determine the current animal health care practices and the needed assistance received by the families in the municipalities of the third district of Cagayan.

The benchmark survey utilized formulated questionnaires randomly distributed to 384, 378, 373, 383, 391, 388 and 395 (95% CI) household respondents from different barangays of Amulung, Enrile, Iguig, Penablanca, Solana, Tuao and Tuguegarao respectively. The household respondents served as the representative samples for the study and the source of the first-hand information for the bench mark survey. All data gathered were collated and analyzed descriptively. Results revealed that 62% of the respondents were aware of the health status of their animals. Seventy one percent (71%) could clearly recognize ill animals yet preferred to resort on other medication and herbal plants (54%) and do self- prescription (28%) rather than go to a veterinarian for advice and consultation. Fifty four (54%) attested that there are regular programs implemented in their community with vaccination (52%) and deworming (39%) as the primary services encountered. Fifty four percent (54%) claimed to have witnessed seminars conducted by other agencies with rabies (57%) as the main issue. Sixty three percent (63%) of the attendees asserted that services had regularly been followed-up by the implementers. Vaccination (37%) still remains to be the main concern and assistance needed by the respondents. As a result of the study, household respondents are very much dependent with the observed repeated activities implemented by some agencies yet remain to be uncertain with their responses and demands. Conclusively, it is apparent that lapses occur in the dissemination of information's that are made critical to the understanding of the community. Strong participation of veterinarians and technical experts should therefore be better emphasized in the program of activities in the community development and a continuing basis to keep tract of unknown barriers that may have caused the failures in implementation.

Key words: benchmark, survey, veterinary, needs, extension

The problems and obstructions on teaching and learning of agricultural subject of agricultural teachers in Secondary School in Northeast Region of Thailand

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The purposes of this study were : 1) to study of problems and obstructions on teaching and learning of agricultural subject of agricultural teachers in secondary school in Northeast region of Thailand, and 2) to compare of the problems and obstructions on teaching and learning of agricultural subject of agricultural teachers with their basic factors. The questionnaire was use to gathering data from 192 agricultural teachers in one month assigning. The data were analyzed by percentage, mean, standard deviation, t-test, F-test, and Scheffe-test. The results were as follows:
1) Most of respondents were male with 68.20%, age above of 50 year old (35.94%), highest academic degree of BS (65.11%) with agricultural area, and they were having teaching experience lower 8 year and between 21-30 years (23.96%) equally. Most of them were teaching in the middle school. The respondents were having trained to enhance their knowledge and improve their teaching more than 3 times (35.42%). 2) problems and obstructions on teaching and learning of agricultural subject of teachers in overall were in the middle level with mean of 3.07. In order 3 their problems and obstructions were follows: 1) classrooms and facilities have not been developed to be suitable for agricultural teaching purposes with mean of 3.32, 2) teachers having too many other commitments besides teaching with mean of 3.31, and 3) the problems of financial support with mean of 3.27. 3) The results of comparison between problems and obstructions on teaching and learning of agricultural subject with the differences of school side, teaching experience, education attaining, and training experiences. The results were as follows:
(1) Teacher who teaching in differences school side having problems and obstructions on teaching and learning of agricultural subject was significant difference at the 0.05 level with extra-large school side difference with small school side. (2) teacher who teaching in differences of school side having problems and obstructions on teaching and learning of agricultural subject was significant difference at the 0.05 level with the interest and responsibilities of students studying agriculture, the problems of teaching and learning preparation, and teachers having too many other commitments besides teaching. In order of teachers who was heaving teaching experiences more than 20 years was difference with teachers who heaving teaching experiences lower than 11 years. Another, the problems of financial support factors show that teachers who heaving teaching experiences more than 20 years was difference with teachers who heaving teaching experiences lower than 11 years. (3) teachers who have difference of training experiences having problems and obstructions on teaching and learning of agricultural subject was non-significant difference at the 0.05 level. (4)

teachers who have difference of education attaining having problems and obstructions on teaching and learning of agricultural subject was non-significant difference at the 0.05 level.

Key words: problems and obstructions, agricultural teacher, teaching and learning, agricultural subject, secondary school

The need for development of content and practical agricultural teaching of agricultural instructors, Institute of Vocational Agriculture in Central Area

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The purposes of this study were : 1) to study of the need for development of content teaching and practical agricultural teaching of agricultural instructors in Institute of Vocational Agriculture in Central Area, and 2) to compare the need of development of content learning and practical agricultural teaching between of the education level of teaching and teaching experiences difference. The respondents were 247 instructors of 280 instructors 88.21)%(who were teaching in agricultural subject. The data were analyzed by percentage, mean, standard deviation, and f-test. The results were as follows: 1) Most of respondents were male with 77.33%, age above of 50 year old (46.56%), highest academic degree of BS (53.85%) with agricultural area, and they were having teaching experience between 21-30 years (31.18%). 2) the need for development of content teaching of agricultural instructors were found that overall was high level with mean of 4.18. The highest level of theirs need were 7 items, and the highest mean item was the content of agriculture subject should be used to applied in living life with mean of 4.50. Another 6 items were high level. 3) the need for development of practical teaching of agricultural instructors were found that overall was high level with mean of 4.09. The highest level of theirs need were 2 items, and the highest mean item was the activities in farm should be the key strategy of the institute with mean of 4.34. Another 5 items were high level.) The results of comparison between content and practical agricultural teaching and the education level of teaching were not significant difference at the 0.05 level. But the comparison between content and practical teaching with teaching experiences were significant difference at the 0.05 level. In order of the Scheffe-test of the difference groups of content teaching were found that: the group of instructor who having experience between 21-30 years was having significant difference at the 0.05 level with group of instructor who having experience between 11-20 years, and group of instructor who having experience lest than 11 years. And the comparison of practical teaching with group of instructors experience were not significant difference at the 0.05 level.

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Key words: need, agricultural instructor, content agricultural teaching, practical agricultural teaching, Institute of Vocational Agriculture

The state of farm factors according to the opinions of high vocational certificate students of Ubonratchathani College of Agriculture and Technology, Ubonratchathani Province, Thailand

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The purposes of this study were: 1) to study the state of farm factors according to the opinions of high vocational certificate students of Ubonratchathani College of Agriculture and Technology, and 2) to compare the opinions of the state of farm factors of high vocational certificate students in deference majors. Sampling group of the research were 110 students of 126 students with 87.30%. The data were analyzed by percentage, mean, standard deviation, One way Anova and Scheffe test. The results were as follows: 1(Most of students were men 50.91)% (and women49.09) %(with mean age of 18.44 year old. They were studying in major of Agro-Industry (31.82%), Animal Science (22.73%), Fisheries (22.73%), Crop Science (12.73%) and *Agricultural* Mechanics (10.00%) respectively. 2) The respondents were having experiences on collage farm practices of fish raising (78.18%) swine farm (60.00%) vegetable farm (46.46%) cattle farm (46.36%) dairy farm (45.45%) orchards (38.18%) mushroom farm (37.27%) hydroponics (35.45) layer farm (32.73%), broiler farm (28.18%) integrated farming and New Theory (22.73%) and ornamental plants (0.91%), respectively. 3) The results of state of farm factors according to the opinions of students in overall were high level ($\overline{X}$=(3.50. In order of their opinions of state on farm factors of each factor were as follows: 1) water resources and water systems used in farm ($\overline{X}$=(3.80, farm area/ location ($\overline{X}$=(3.72, personnel and farm labor ($\overline{X}$=3.72(, plant and animal breeds ($\overline{X}$=(3.56, and farm management ($\overline{X}$=(3.48. In addition, their states of moderate level of farm building and housing ($\overline{X}$=3.23(and tools and equipment use in farm ($\overline{X}$=3.00(, respectively. 4) The results of comparison on state of farm factors according to the opinions of students who are studying in deference major by using One Way ANOVA found that overall of opinion was significant difference at the 0.05 level. In order of considering with each major area was found significant difference at the 0.05 level with major area were as follow: 1) farm area/ location 2) water resources and water systems used in farm, 3) farm building and housing, and 4) tools and equipment use in farm. In order of

comparison with each major of students using the Scheffe test was found the overall that the opinion of Animal science students was significant difference at the 0.05 level with the opinion of Crop Science students, *Agricultural* Mechanics students, Agro-Industry students, and Fisheries students. In addition, the comparison with each factor was found the results as follow: (1) Farm area/ location : Fisheries students state of farm factors according to their opinions was significant difference at the 0.05 level with *Agricultural* mechanics students and Crop science students, another Agro-industry student and Animal science students state of farm factors according to their opinions was significant difference at the 0.05 level with Crop science students. (2) Water resources and water systems used in farm: *Agricultural* mechanics student's state of farm factors according to their opinions was significant difference at the 0.05 level with Crop science students.(3) Farm building and housing: Animal science student's state of farm factors according to their opinions was significant difference at the 0.05 level with Crop science students.(4) Tools and equipment use in farm: Animal science student's state of farm factors according to their opinions was significant difference at the 0.05 level with Crop science students, *Agricultural* mechanics students, Agro-Industry students, and Fisheries students.

Keywords: student's opinion, agricultural farming, state of farm factors, high vocational certificate student

Factors affecting the cognitive competency of the pre-school children in selected day care centers in Diffun, Quirino, Philippines

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This study was conducted to uncover the factors that affect the development of the cognitive competency during the pre-test and post-test of the preschool children from the selected day care centers in Diffun, Quirino. It aimed to describe the socio-demographic profile of the preschool children and their performances in the cognitive competency during the pre-test and post-test. It also determined the significant relationships/differences on their performances on the said competency during the pre-test and post-test and their socio-demographic profile. Also, factors that affect the competency were identified. Furthermore, this study examined which among the factors have significant relationships on the performances of the preschool children during the pre-test and post-test. There were 420 preschool children, ages 3 to 5 years old from the selected day care centers in Diffun, Quirino as respondents in this study. The researcher made use of descriptive method and inferential statistics like t-test, Spearman Rho correlation, and Stepwise Regression to test the hypotheses. The data were gathered using the Early Childhood Care and Development Checklist. The data revealed that majority of the respondents are "Competent" in most of the cognitive competencies during the pre-test and became "Very Competent" during the post-test when they are grouped according to their socio-demographic profile. There are items that have significant relationships/differences on the

cognitive competency during the pre-test and gender, and birth order of the respondents. Significant relationships/differences were also noted on some items on the cognitive competency during the post-test and the respondent's number of siblings, and birth order. Moreover, school, social and family parameters are identified to find out which among these affect the performances of the respondents on cognitive competency during the pre-test and post-test. The result of this study revealed that majority of the school factors affect the performance of the respondents on the said competency during the pre-test and post-test while the social and family factors have lesser effects. Thus, this study implies that continuous partnership of the school and home is essential, more organized activities has to be implemented and strict monitoring and evaluation of school program is needed in the Day Care Centers to improve further the cognitive competency of day care children.

Key words: cognitive, early childhood, day care center

The need of students and student's parents on development of learning and teaching agricultural **subjects of Phraibueng Wittayakhom** School, Sisaket Province

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The purposes of this study were: 1) to study of the need of students and student's parents on development of learning and teaching agricultural subjects of Phraibueng wittayakhom school, Sisaket province, and 2) to compare the need of development of learning and teaching agricultural subjects between students and student's parents. Sampling group of the research was 163 students who study on agricultural subjects and 170 student's parents, selected by using Simple Random method. The data were analyzed by percentage, mean, standard deviation, and t-test. The results were as follows: 1) the need of students on development of learning and teaching agricultural subjects in 5 aspects including (1) subject content, (2) teacher, (3) media and equipment, (4) measurement and evaluation, and (5) other aspects. In overall that students were need of development of learning and teaching agricultural subjects at a high level. The comparison between students based on family farming and non-family farming found that the need for development of teaching agriculture in general and the difference is statistically significant at the 0.05 level, except for the measurement and evaluation aspect were no significant difference statistically significant.2) the need of student's parents on development of learning and teaching agricultural subjects in 5 aspects. In overall that student's parents were need of development of learning and teaching agricultural subjects at a high level. The comparison between student's parents based on family farming and non-family farming were found that the need for development of teaching agriculture in general and

the difference is statistically significant at the 0.05 level, except for the subject content aspect were no significant difference statistically significant.3) comparison of need of development on learning and teaching agricultural subjects between students and student's parents were found that in both of overall and each aspect were high level.

Key words: need, learning and teaching, agricultural subjects, high school student, student's parent

Satisfaction of **high vocational certificate student on** training in **farm practices** of Ubonratchathani College of Agriculture and Technology, Ubonratchathani Province, Thailand

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The purposes of this study were: 1) to study of satisfaction of high vocational certificate student on training in farm practices of Ubonratchathani College of Agriculture and Technology, and 2) to compare the satisfaction of high vocational certificate student on training in farm practices in deference majors. Sampling group of the research were 110 students of 126 students with 87.30%. The data were analyzed by percentage, mean, standard deviation, One way Anova and Scheffe test. The results were as follows: 1(Most of students were men 50.91)% (and women 49.09)%(with mean age of 18.44 year old. They were studying in major of Agro-Industry (31.82%), Animal Science (22.73%), Fisheries (22.73%), Crop Science (12.73%) and *Agricultural* Mechanics (10.00%) respectively. 2) Students were state of high level of satisfied with the training in farm practices with 7 major areas: (1) training on the college's farm of (2) plant and animal breeds, and (3) water resources and water systems used in farm (4) personnel and farm labor (5) farm management (6) farm area / location, and (7) tools and equipment use in farm, and they were moderate satisfied with farm building and housing. 3) The results of comparison of the satisfaction with training in farm practices of students who are studying in deference major by using One way Anova found that overall of satisfaction with the training in farm practices was not significant difference. In order of considering with each major area was found the significant difference at the 0.05 level of the level of satisfaction with major area were as follow: 1) water resources and water systems used in farm, 2) farm building and housing, 3) personnel and farm labor 4) farm management, and 5) training on the college's farm. In order of comparison with each major of students using the Scheffe test was found the results as follow: (1) The major area of water resources and water systems used in farm: the major of Fisheries students was satisfied with training in farm practices with significant difference at the 0.05 level with students in major of *Agricultural* mechanics. (2) The major area of farm building and housing: the major of Animal science students was satisfied with training in farm practices with significant difference at the 0.05 level with students in major of

Crop science. (3) The major area of personnel and farm labor: the major of *Crop science* students was satisfied with training in farm practices with significant difference at the 0.05 level with students in major of *Animal science and major of Agricultural* mechanics. (4) The major area of farm management: the major of *Agricultural mechanics* students was satisfied with training in farm practices with significant difference at the 0.05 level with students in major of Fisheries. *In addition, the major of Crop* Science students was satisfied the training in farm practices with significant difference at the 0.05 level with major of Fisheries.

Keywords: satisfaction, agricultural farming, training in farm practices, high vocational certificate student

Psychological strengths and emotional difficulties of students with separated parents: A basis for counseling intervention program

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The study was conducted primarily to determine the Psychological Strengths and Emotional Difficulties of freshmen students of Quirino State University, Diffun, Quirino for school year 2014-2015. Frequency counts and percentages on the description of the profile of the student-respondents in terms of gender; age; birth order; number of siblings; parents' educational attainment; parents' occupation; and absent parent; means on descriptions of the respondents' psychological strengths and emotional difficulties. The study conducted is a descriptive type of research which enabled the researcher to determine and describe the Psychological Strengths and Emotional Difficulties of the respondents. The findings are used in the pursuit of implementing a counseling intervention program with emphasis on respondents' psychological strengths in resolving the emotional difficulties of the respondents. The respondents' psychological strengths and emotional difficulties were determined using an adopted questionnaires consisting of 35 items for psychological strengths while 20 items for \emotional difficulties. The data were subjected to appropriate statistical tools using computer application. It was found out that (1)The respondents of the study are mostly females with the age of 16, are first born children and from big family with 4 and above number of siblings; (2) The parents' educational attainment is mostly high school graduate; (3) The occupations of the fathers are mostly self-employed while the mothers are OFW; (4) Most of the respondents are living with their grandparents and relatives since parents' separation; (5) In general, the respondents' psychological strengths are revealed in all categories. However, the Cognitive and Appraisal Skills has obtained the highest mean followed by Interpersonal Skills and Supports with which the outlook for strength based counseling intervention is seen better to process; and (6) the most common emotional difficulty among the respondents was revealed along peer problems.

Key words: psychological strengths, child, parents

POSTERS

Cultures of fairy shrimp (*Streptocephalus sirindhornae* Sanoamuang et al., 2002) for feeding giant freshwater prawn [*Macrobrachium rosenberbii* (De man,1879)]

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The adult fairy shrimp (*Streptocephalus sirindhornae* Sanoamuang et al., 2002) of 0.5, 1.0 and 2.0 centimeters in length was designed as food for different age groups of giant freshwater prawn [*Macrobrachium rosenberbii* (De man,1879)] to evaluate size and number of fairy shrimps and suitable ratio of fairy shrimp to substitute pellet feed for feeding giant freshwater prawn, respectively. Four age groups of giant freshwater prawn: a group beginning reared in earthen pond (0 month old), a group reared in earthen pond for 1, 2 and 4 months old were reared in aquarium tanks for 7 days. Completely Randomized Design (CRD) with 3 replications of 3 sizes of fairy shrimp: 0.5, 1.0 and 1.5 centimeters in length were tested. Three age groups of giant freshwater prawn of 1, 2 and 4 months old were reared in aquarium tanks for 45 days. Completely randomized Design (CRD) with 3 replications of 5 food rations of fairy shrimp to pellet feed: 100:0,75:25,50:50,25:75 and 0:100 were tested.

The results showed that fairy shrimp of 0.5 centimeters in length can be used as food for giant freshwater prawn of age 1 month and older. The optimal size of fairy shrimp for feeding giant freshwater prawn of age 1, 2 and 4 months were 0.5, 0.5-1.5 and 0.5-1.5 centimeters in length, respectively. The number of fairy shrimp fed by giant prawn of age 1, 2 and 4 months were 32.09, 32.86-43.57 and 24.3-33.27 individuals per day, respectively. Moreover, fairy shrimp could be used to substitute pellet up to the ratio of 100%.

Key words : fairy shrip, prawn, culture

The competency model of engineer in the automotive industry: The data was collected from Amata Nakorn Rayong

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This research was aimed to create a competency model of engineer in the automotive industry. The data is collected from Amata Nakorn Rayong. The research is performed with two steps: (1 create an engineering competency in the automotive industry using Delphi technique with 17 experts. Then 2) review the engineering competency with the focus group of management staffs from automotive industry in Amata Nakorn Rayong. The statistics used in the analysis were percentage, median and interquartile range. The results show that 1(an engineering competency is consist of three parts. Part 1: Management Competency consists of: 1) the management of operational performance, Part 2: Functional Competency consists of 2) human resource management 3) project management and Part 3: Production Engineering characteristic consist of 4) personal characteristics.

Keywords : Engineering Competency Model ,automotive industry

Studying of plant materials on releasing available nutrients

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This study aimed at researching on effect of plant materials on releasing available macronutrients. Completely randomized Design was used as an experimental design consisted of 6 treatments and three replications, i.e., T1 : bare soil (control), T2 : soil + cattle manure, T3 : soil + charcoal, T4 : soil + peanut, T5 : soil + leaf compost and T6 : soil + cattle manure + charcoal + leaf compost + peanut shells. Used in the ratio of 1:1 by volume, soil and plant material and analysis of major nutrients (N P K) released from different plant materials. The test plant was broccoli. Result revealed that plant materials and plant nutrients in T4 (soil + peanut) as follows; pH (6.94), Electricity conductivity (0.09 dS/m), organic matter (0.55%), total nitrogen (0.02 g/kg), available Phosphorus (36.64 mg/kg) and available Potassium of 51.38 mg/kg. Therefore broccolis at harvest (63 days after planting) are growing most flowers and fresh weight of 12.63 cm/flower and 183.23 g/flower, respectively. The results did not difference with the plant material is fermented leaves. While T2 (soil + cattle manure) mineralization least make a useful plant nutrient uptake was slow. The yields were smaller.

Key words: plant materials, macronutrient, available nutrients

Screening and potential of some plant extracts on growth of chilli anthracnose

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The Screening and potential of some plant extracts on growth of chilli anthracnose, caused by *Colletotrichum* spp. was examined. The crude extracts of root and leaf of *Curcuma longa* L., *Psidium guajava* L. and *Punica granatum* L., which extracted with 95% Ethanol, 40% Ethanol and distilled water, were screened to determine their effect on the mycelial growth and sporulation of *C. gloeosporioides* C01 using poisoned food technique. Results showed that all crude extracts could inhibit mycelial growth of *C. gloeosporioides* C01 except the crude extract of *C. longa* L. which extracted with distilled water. However, all crude extracts could inhibit sporulation of *C. gloeosporioides* C01. The crude extract of *C. longa* L. which extracted with 95% Ethanol presented the best result for the inhibition of sporulation at the concentration of 3,000 µg/ml. It is proved that the crude extracts of root and leaf of *Curcuma longa* L., *Psidium guajava* L. and *Punica granatum* L. become the promising for inhibition plant pathogenic fungi.

Key words: anthracnose, plant extract, biological control

The efficacy of herbicides on weed control in oil palm plantation

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The experiment on the efficacy of herbicides on weed control in oil palm plantation was conducted in 4-year- old oil palm plantation at the faculty of Agriculture, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, Thailand from January to March 2013. The experiment design using RCBD with 3 replications and 7 treatments : control (untreated), brush cutter, paraquat at the rate of 110.4 g/rai, glyphosate at the rate of 82 and 123 g/rai, glufosinate-ammonium at the rate of 60 and 90 g/rai. The results showed that the effective weed control herbicides for 45 days after application were glufosinate- ammonium at the rate of 90 g/rai with the highest percentage of the weed killed at 78.33 %, followed by glyphosate at

the rate of 123 g/rai, glufosinate–ammonium at the rate of 60 g/rai and glyphosate at the rate of 82 g/rai with no significant differences in the percentage of the weed killed at 60.00 56.67 and 53.33 % respectively. Paraquat at the rate of 110.4 g/rai and brush cutter were good weed control for 15 days with the percentage of the weed killed at 66.67 and 53.33 % respectively and those applied for 45 days the efficacy were 26.67 and 6.67 % respectively.

Key words : paraquat, glufosinate-ammonium, glyphosate, brush cutter

Collection and evaluation of local Thai rice varieties)*Oryza sativa* L.(

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This study explored the biodiversity in Thai native rice during May 2011 to March 2013 by conducting surveys and collecting rice varieties from 4 regions of Thailand. There are altogether 89 varieties of native rice which include 16 in central region, 12 in northern region, 23 in northeastern region, and 38 in southern region. Among them are of 2 types: 21 varieties of glutinous rice and 68 varieties of non-glutinous rice. Ecological classification suggested 72 varieties of lowland rice, 10 varieties of upland rice, and 7 varieties of floating rice These varieties of rice were cultivated in the farmer fields of Nongbondaeng sub-district in Banbueng district of Chonburi province during the rainy season and off season between 2011 and 2013 and to capture their agricultural descriptors. Results revealed germination and seedling growth in 35 varieties. The Unknow II variety yields maximum weight of 6.47grams per panicle, while Sor 6Chumpae variety gives minimum weight of 0 85.grams per panicle. Ai-Kong produces highest number of 317 seeds per panicle, while the lowest is Sewkliang with 51.33seeds per panicle. Gumrai gives biggest size of seed of 0.47x 1.01mm., while Sangyod gives smallest size of 0.19x 0.97mm.

Key words: rice, Thai varieties, panicles

Screening Chaetomium spp. as antagonist against *Colletotrichum gloeosporiodes* and *Pythium aphanidermatum* causing diseases of pomelo

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Two isolates of plant pathogenic fungi were isolated from leave anthracnose and root rot of pomelo. The isolates were morphologically identified as *Colletotrichum gloeosporiodes* (CL01) causing anthracnose, and *Pythium aphanidermatum* (PY.S02) which causing root rot of Pomelo. *Chaetomium cupreum*, *Chaetomium globosum* and *Chaetomium lucknowenese* as antagonist, which respectively inhibited 30.69, 37.78 and 34.86 % of colony growth and 51.71, 70.10 and 60.54% % of spore production of *C.gloeosporiodes* CL01when compared with the control. While, all these antagonists completely grown over the colony of *P. aphanidermatum* (PY.S02) in bi-culture plates at 30 days, and inhibited 53.89, 86.41 and 89.01% of spore production of the pathogen. This research finding indicated that *Ch. lucknowenese* is reported for the first time to inhibit *C. gloeosporiodes* causing anthracnose of Pomelo. Further study would investigate on their fungal metabolites against these pathogens and also test *in vivo*.

Key words: anthracnose, pomelo, metabolites

Enhancing value-added products from the endangered mabolo fruit *(Diospyros blancoi)*

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Mabolo or Kamagong is an indigenous Philippine fruit which is raised commercially in small scale. There are several uses for the Mabolo plant and fruits because of its commercial value. Aside from its ornamental significance, it is very useful for its nutritional benefits and medicinal functions. However, in Quirino province many people do not see the value of the fruit and is rarely eaten nor commercially sold in the market for consumption. Thus, the development of wine, jam and pickles out of mabolo is a very good strategy to introduce the essence of the fruit and for the locals to appreciate its value. The highlights of the project includes the processing of wine through starter preparation, must preparation, treatment of the "must", fermenting, harvesting, storing, and aging. A kilo of mabolo pulp can produce four (4)

bottles of wine at 750 mL for PhP 180.00 per bottle. The mabolo jam from a kilo of mabolo pupl can produce six (6) fancy oval jars sold at PhP 50.00 per bottle. It follows the process of washing, measuring, blending, mixing, cooking and bottling. Moreover, a kilo of unripe mabolo can produce four (4) jars of mabolo pickles at PhP 40.00 per jar. It undergoes the process of washing, soaking, slicing, preparing the brine and pickle solution, draining, preserving and sealing. The processing of Mabolo products are done at the food processing center of Quirino State University. Before the product is made available commercially, it undergoes sensory evaluation as to the acceptability of the products to assure viability of the products in the market. It is now a growing small scale enterprise and income generating project of the university.

Key words: mabolo, wine, jam, pickles

Morphological and genetic diversity of *Fusarium oxysporum* isolated from Thailand

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The genus *Fusarium* included many species that caused plant disease, such as vascular wilt, root stalk, collar rot of seedling, and rot. This study aimed to evaluate the diversity of *Fusarium* species based on morphological characteristics and ISSR marker. Twenty five isolates of *Fusarium* were isolated from different plant disease in Thailand. These isolates were cultured on Potato Dextose Agar (PDA), Spezieller-Nahrstoffar Agar (SNA), Malachite Green Agar (MGA), and Peptone PCNB Agar (PPA). Twenty five isolates of *Fusarium* were identified as *F. oxysporum, F. semitectum* and *F. solani* using colony type, colony pigment, macro conidia and microconidia characters on different media. Moreover, all isolates were observed for genetic diversity using ISSR marker with $GCG(CAG)_5$, $(CAG)_5$, $(GTG)_5$ primers. The Results showed that there was a high polymorphic DNA among *Fusarium* species. The results indicated that there were genetic diversity within and among species of *Fusarium*.

Key words : *Fusarium* sp., macroconidia, microconidia, chlamydospore, inter simple sequence repeats (ISSR) marker

Evaluation of alternative trap from sap of selected trees as bio-pesticide to control mice

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The main objective of the study was to formulate sticky mouse trap for the control of house mice. It aimed to determine the efficiency of each formulated mouse trap from selected sap of trees in trapping mice. Mousetrap and bio pesticide play an important role in sustaining healthy environment in everyone's home by controlling pest like rats that can cause a severe infestation if not treated properly.In the past decade, leptospirosis has emerged as a globally important infectious disease. It occurs in urban environments of industrialised and developing countries, as well as in rural regions worldwide. No vaccine is available. Prevention is largely dependent on sanitation measures that may be difficult to implement, especially in developing countries. There were 4 treatments with three replicates. The treatments were T1- Mango sap, T2- Jackfruit sap, T3- Acacia sap and T4- Commercial sticky Mouse trap. Based from the result the sap from mango is significantly effective as mouse trap having 2.67 comparing to the commercial mouse trap which got 3. This implies that sap from mango can be used to replace the high cost of commercial mouse trap with added benefit because natural sticky trap from trees are human and eco-friendly. Percent of mice trap in the sticky trap was significantly influenced by the different treatments on the first day of observation. At 5% level of significance, DMRT revealed that significantly the highest percent being trap in the sticky trap is the mango sap comparing the commercial mouse trap. Jackfruit also is significantly effective as trap comparing to the mango sap. Mango saps are comparable with that of the commercial sticky trap, bio pesticide sticky trap from mango can be used instead of the commercial one. Less cheap and can be easily prepared. The researchers concluded that sticky trap from sap of Mango has the greater potential in controlling mice followed by the sap from Jackfruit. It was also concluded that this sticky trap from Mango can be used as substitute to the commercial mouse trap because of the comparable percent obtained with that of the commercial trap used.

Key words: mouse trap, sap, bio-pesticide

Effect of intercropping between upland rice and mungbean for soil amendment under the limited growing season

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This research aimed to study soil improvement by amending soil with organic manure (mungbean) in field where plant had been cultivated once per year during the rainy season at Nong-Ya-Plong, Phetchaburi province, Thailand. Intercropping between upland rice and mungbean in field was used as model for study the effect of intercropping between major plant and legume plant on soil fertility and others in the non-irrigated area. For intercropping, yield of upland rice and mungbean were 356.25 and 1,681 kg/ha, respectively under not supplemented both of inorganic and organic fertilizers. Plant height of some upland rice plants had been improved when they were planted close to mungbean plots. However, the score of infestation of diseases (such as Bacterial leaf blight disease and Dirty panicle disease) also had been increased. Nevertheless, after cut and incorporated both of upland rice and mungbean residues into the soil, the organic matter and total nitrogen in soil amended with mungbean residue was higher than that amended with upland rice residue.

Keywords Organic soil amendment, Water shortage, Non-irrigated area, Soil fertility

Comparison on rice cultivation applying chemicals, good agricultural practice and organic methods in the field in Lao PDR

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Drechslera oryzae causing shealth blight of rice and *Curvularia lunata* causing brown leaf spot of rice were isolated and proved that the potent of *Chaetomium cupreum* CC03, *Chaetomium globosum* Cg05, *Chaetomium lucknowense* ChL that reduced inoculum production of D.

oryzae and C. lunata. Result showed that organic, GAP and chemical methods cultivated in the field in rice var Khao Jao Mong (KJM) and Tha Dork Kham-11 (TDK-11) gave significantly better in growth parameters or plant stands than the non-treated control. Rice var Khao Jao Mong (KJM) cultivation gave significantly highest yield in GAP method (27.27 kg) and followed by organic and chemical methods (26.42 and 26.25 kg., respectively) when compared to the control (21.67 kg). Rice var Tha Dok Kham-11 (TDK-11) cultivation revealed that chemical method gave significantly highest yield (23.90 kg) and followed by GAP and organic methods (22.24 and 21.97 kg., respectively) but significantly differed when compared to the control.

Keywords: shealth blight of rice, *Chaetomium* spp.

Survey collection, isolation and morphological identification of mushrooms in Chanthaburi Province of Thailand

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The research findings in this study, all specimens were collected in Krating Waterfall , Chanthaburi Province , Thailand which morphological identified into 26 species. As follows: *Agaricus* spp ,*Clavulinopsis fusiformis, Clavulinopsis helvola, Clitocybula atrialba, Collybia dryopjila, Lactarius controversus , Lactarius sanguifluus , Laccaria* spp (CH3-1), *Laccaria* spp (CH3-13), *Laccaria* spp (CH3-24), *Laccaria* spp (CH3-27), *Marasmiellus albuscorticis, Marasmius androsaceus, Marasmius foetidus, Marasmius oreades, Marasmius plicatulus, Marasmius scorodonius, Marasmius* spp, *Mycena inclinata, Mycena rosella, Mycena subcaerulea, Resinomycena rhododendri, Termitomyces microcarpus, Trametesversicolor* spp, *Tremiscus* spp, *Tricholomatacea* spp, *Xylaria hypoxylon.*

Key words: survey, collection, mushroom

Bacteriostatic properties of different food based essential oils

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Essential oils contain active ingredients that have potential antimicrobial properties that may be harnessed for controlling bacterial infections. The antibacterial properties of the different food based essential oils, namely, red thyme, lemon, peppermint, eucalyptus and rosemary were evaluated in this study. The disk assay protocol was used in determining the inhibitory properties on the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Escherichia coli*. The zones of inhibition were measured and compared. Results of the study showed that essential oil of red thyme significantly inhibited the growth of all the organisms tested . Essential oil of Eucalyptus produced zones of inhibition against *S. aureus, B. cereus and Escherichia coli* but not *P. aeruginosa*. Essential oils of rosemary and lemon were able to inhibit the growth of S. aureus, *P. aeruginosa, B. cereus* but not *E coli*. Peppermint essential oil did not inhibit the growth of *S. aureus* but did the other organisms. It was also noted that red thyme did not only inhibit the growth of all the test organisms, but it also produced the biggest zones of inhibition.

Key words: antibacterial, essential oils, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Escherichia coli*.

Two powdery mildew fungi on *Euphorbia heterophylla* in Thailand

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The accurate identification of powdery mildew fungi (Ascomycotina, Erysiphales) is desirable, not only to determine appropriate control measures, but also for breeding resistance among agricultural crops and to ensure that quarantine barriers are not broken. During a survey of Erysiphales in the northern and southern part of Thailand, two powdery mildew fungi; *Ovulariopsis* sp. and *Oidiopsis* sp. were found on *Euphorbia heterophylla*. Interestingly,

powdery mildew fungus on *E. heterophylla* has been recorded only *Oidiopsis* sp. in previously time. This is the first report of *Ovulariopsis* on *E. heterophylla* in Thailand.

Key words: Erysiphales, taxonomy, *Ovulariopsis* sp., *Oidiopsis* sp. and *Euphorbia heterophylla*

Survey of rice dirty panicle fungi in Thailand

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Rice (*Oryza sativa* L.), is a cereal grain, and economic crop in Thailand. The dirty panicle of rice is the most important disease of rice production in Thailand. Rice dirty panicle cased by six fungal pathogens such as *Curvularia lunata Bipolaris oryzae Cercospora oryzae Trichoconis padwickii Sarocladium oryzae* and *Fusarium semitectum*. Rice infection by fungi decreased seed germination, quality and quantity of rice production. The aim of this study to evaluate the epidemiology of dirty panicle fungi in the rice paddy fields in Thailand. The disease sample were collected from Cheng rai Pitsanulook Sukhothai Phetchaboon Aungtong Suphanburi and Nakhonphatom provinces. Then, fungal isolation and morphological observation was done in the laboratory. The seed symptom was divided into 7 levels. The results showed that the symptom level 4 (26-50% of spot on seed) was found with 70 % highly. The morphological based identify revealed that four species were observed and identified as *Curvularia lunata* (53%) *Fusarium semitectum* (39%) *Helminthosporium oryzae* (7%) and *Trichoconis padwickii* (1%). The results indicated that there were different fungal species caused the dirty panicle disease in Thailand.

Key words: rice dirty panicle, *Curvularia lunata, Bipolaris oryzae, Cercospora oryzae, Trichoconis padwickii, Sarocladium oryzae, Fusarium semitectum*

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Sustainable development of finance, accounting and marketing for producing Sangyod Muang Phatthalung rice (GI rice) in Phatthalung Province

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The research of sustainable development in finance, accounting and marketing for produce Sangyod Muang Phatthalung rice (GI rice) in Phatthalung province has objectives to 1) examine the cost and return of cultivation for songyod muang phatthalung rice of small farmers, 2) Study of marketing of songyod muang phatthalung rice and 3) develop a accounting system for processing group of songyod muang phathalung rice. The researchers divided the sample target into two groups such as 1) A total of 80 people; small farmers who cultivate songyod muang phathalung rice and 2) A total of 5 processing groups of coarse rice songyod muang phathalung by obtained interviewing form for small farmers who cultivate songyod muang phathalung rice and processing groups of coarse rice songyod muang phathalung. From result of small farmers who cultivate songyod muang phatthalung rice found that 1) the total cost on a year per acre was equal to average of 4,850.33 baht which divided into an average fixed cost for a year per acre was equal to 940.00baht, representing as 19.38 percentage and average variable cost for a year per acre was equal to 3, 910.33baht, representing as 80.62 percentage. 2) An average return for a year per acre of cultivate songyod muang phatthalung rice composed of a total average income for a year per acre was equal to 7,614.00 baht and net income for a year per acre was equal to 2,763.67 baht, representing as 36.30 percentage and 3) Marketing of songyod muang phatthalung rice consist of sell pattern that The most of sole paddy in field was equal to 57.50 percentage; The most of distribution channels sole to mill or processors was equal to 80.00 percentage; Setting of selling price was used market price. It was equal to 58.75percentage; the most of payment of cultivator for trading use immediate payment as sold. It was equal to 98.75percentage and the buyers arranged grain shipment by themselves with representing as 96.25 percentage. The result of processing groups of coarse rice songyod muang phathalung shown that the researchers have developed an accounting system to the groups which a setting suitable accounting system for groups. They have an effective internal control in order to prevent the loss of property. The accounting system is divided into six elements for supporting accounting procedures such as 1) the organizational structure, 2) accounting chart, 3) account books, 4) accounting documentations, 5) the model of financial reporting, and 6) bookkeeping and financial statements

Key words: sustainable development, songyod muang phatthalung rice

Cost management strategy for small and medium enterprises in rubber industries, Upper Southern Region, Thailand.

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The best performance of cost management strategy for small and medium enterprises in rubber industries, upper southern region, Thailand was the objective of this research. The important key performance of small and medium enterprises in rubber industries were analyzed and implied. Small and medium enterprises in rubber industries, upper southern region generated a sample size from 249 enterprises, which data was collected realistically though 155 enterprises participated by Yamane. Three of boards for each 155 enterprises were interviewed as overall 465 interviewees. The result revealed that the best performances of the cost management strategy of marketing were the quantity discount consumer, reducing sale and marketing staff salary and the price of competitor monitoring. The best performances of the cost management strategy of financing were new source of finance for expansion and low interest rate loan. The key performance of the cost management strategy of managing ere salary rate of staff related to their working ability, office equipment maintenance on time to reduce cost and economical and cost shipping control. Finding of low price and high quality of raw material, paying salary and wage on ability and workloads, choosing high quality of shipping and electric cost control were the key performance of the cost management strategy of product managing.

Key words: cost management strategy, small enterprises, medium enterprises, rubber industries.

Application of *Chlorophyllium molypdites* (Meyer ex Fr.) Mass. (PH01) to control *Pestalotia* sp.

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Bioactivity tests of these crude extracts against *Pestalotia* sp. causing disease of tea. Result showed that crude extract of C*hlorophyllium molypdites* (Meyer ex Fr.) Mass. (PH01) at concentration of 1000 ppm. gave the highest percent inhibition of colony of *Pestalotia* sp.

which 21.25, 30.75 and 38.25 percent, respectively with this, percent inhibition of spore production were 78.55, 68.94 and 85.39 percent, respectively.

Key words: crude extract, tea

Botanical product formulations for insect pest management on organic vegetable production

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This study aimed of developing insect pest management product for organic vegetable production enterprise utilizing locally available plant materials in the Region. Specifically, 1). to screen/evaluate different plant materials on selected insect pests, and formulate products for organic vegetable crops in the region, and 2). to determine the economic feasibility of the developed formulations/technologies in organic vegetable crop production system.
Seven formulations against target insect pests were developed from series of laboratory bioassay and field testing. Four were identified potential for on-station verification. The formulations were combination of two or three plant materials such as (F1- Curphora (*Curcuma longa + Tinospora rumphii*), F2, Tacuma (*Tagetes erecta + Curcuma longa,* F3, Glicum (*Gliricidia sepium + Capsicum frutescens*), and F4- Glitap *(Gliricidia sepium+ Tinospora rumphii + Capsicum frutescens).* Convergence trials were conducted using the recommended organic vegetable seeds, solid and foliar fertilizers to develop an organic production system. These formulations/technologies were validated on-station at the RM-CARES experimental area and on- farm with the organic farmers in the region. The organically-grown pole sitao, tomato, amargoso, and eggplant had higher total production than using farmer's practice and the net income increased to 11.96%, 20.0%, 31.9% and 13.26%, respectively. Such findings can be recommended as strategies for successful insect pest management for organic vegetable production in the area.

Key words: insect pest management, formulations, convergence trials.

The strategies of economics development in communities in Thailand and Lao PDR

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This research is conducted to study about the strategies of economics development in communities, in Thailand and Lao PDR which set the objectives with three main points. (1) To study the current difficulties of developing economics in communities in Thailand and Lao PDR (2) to study and seek the strategies of developing economics in communities in Thailand and Lao PDR (3) to build economics development networks in Thailand and Lao PDR It mainly focuses on research and development which studies and asks opinion from local communities to get information as Ethnographic research and this research is analyzed by using SWOT (weaknesses , opportunities and threats) to figure out its result. In addition, It manages locals into groups to interview and conversation in group. The researchers participate in conversation to observe key informants who take part in conversation and neglect to provide information. Sample groups of this research we focus on 40 participants in which 20 are Thai and 20 are Lao, We decided to choose participants of two countries equally for indicating outcomes of the research. According to the research we found that: 1. The current difficulties and obstacles of developing economics in communities in Thailand and Lao can be classified into six main points that are main problems of achieving the goals of improvement. (1). Mostly commodities in markets are agricultural products that use local materials to produce. (2) Funds of producing mostly are land, laboring, material resources and intelligence of local people. (3). Workforces of producing is the member of household. (4). Mostly workforces of producing are households and local people. (5). Natural resources are used for producing products. (6). The cost of selling products is not too high by considering to marketing prices. As a result, manufacturers can take products of low prices and can produce new products with higher costs. 2.From the research we found that strategies of developing economics in communities in Thailand and Lao PDR should include six strategies. (1). Economics innovation should be sustainable. (2). Local community should cooperate and participate activities. (3). Local productions should be value - added. (4). Modern technology in production should be used to increase quantity and quality. (5). Building sustainable community network. (6). Community Economics development both Thailand and Laos should be Locals Model. 3. Network building of economics development in Thailand and Laos have three significant networks. (1). Member network of occupations such as merchants, government officials, researchers, engineers and etc. (2). Geographical networks that categorize activities in each region such as agricultural networks, academic network and etc. (3). The Member of networks is able to exchange information about / on setting objective of economics networks , occupational training and marketing.

Key words , Strategy , Development , Community economics

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Fertility assessment and mapping of rice areas under the Magat River Integrated Irrigation System in Region 02, Philippines

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Soil is important in crop production. It holds water, microorganisms and essential nutrients needed by plants. In the rice areas irrigated by Magat River Integrated Irrigation System (MRIIS) where 2-3 croppings a year is possible, depletion of the native nutrients in the soil becomes a problem. The addition of nutrients, particularly NPK, in the form of inorganic fertilizer becomes a necessity. The immediate effect of inorganic fertilizer lure farmers to apply fertilizers indiscriminately. Hence commercial fertilizers account for approximately 26% of the total cost of rice production. Farmers would borrow against their projected earnings just to buy commercial fertilizers. The result in terms of yield, however, is not always satisfactory. There are times when the potential yield of rice is not attained despite heavy fertilizer application. In view of this, soil and fertilizer management are given much attention. The fertility status of the soil must be known first through soil analysis in order to achieve precision in fertilizer application. This prevents wastage of resources and environmental pollution since excess fertilizer applied will only find their way eventually to bodies of water. In 2003 to 2005, massive soil sampling and analysis was done in the MRIIS area covering some 84,901.832 hectares. Results of analysis showed that of the total area covered, 74.8 % is low (0 to 2.0 %); 22.5 % is medium (2.1 to 3.0%); 2.7 % is high (3.1% above) in OM. For phosphorus and potassium, 13.6% and 24.4% is sufficient in P (above 20 ppm P) and K (above 60 ppm K) respectively. For the micronutrient zinc, 70.3 % is deficient (below 1.15 ppm Zn). Using the soil analysis data, fertility maps for each municipality under the MRIIS area were generated. This will serve as ready reference for farmers to base their fertilizer application even if they did not subject their rice fields individually for soil testing. These maps also serve as guide for the DA management in decision making for the allocation of site specific interventions like biofertilizers, zinc sulfate and other soil enriching materials. Every three years, these fertility maps are updated since the validity of soil analysis results is 3 years or 6 cropping seasons.

Key words: Soil fertility, potential yield, fertility map

Sagitta morphology of freshwater fish in Order Osteoglossiformes and Order Anguilliformes from Southern of Thailand

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Freshwater fish in two Order, (Order Osteoglossiformes and Order Anguilliformes); three family [Osteoglossidae (Bonytongue), Notopteridae (Featherback), Anguillidae (Ture Eel)] and four species (*Scleropages formosus*, *Notopterus notopterus, Chitala ornate, Angguilla bicolor*) were collected from southern of Thailand. Sagittae were extracted from skulls of fish, cleaned and stored dry in vials. Morphology of these sagittae were studied by scanning electron microscope. The sagittal otoliths show clearly different morphology, which is species-specific, e.g. shape, ostium, cauda, rostrum, antirostrum. Other characteristics of sagittal otoliths, e.g. dorsal depression, ventral depression and margin sculpturing are also species-specific. However, morphology of sagitta in same family was similar. The differences in sagittal otoliths morphology might result from environmental, biological factors and the influence of the physiological mechanism in otoliths, which is very important for regulating the otolith growth. The similarity in morphology of sagittae may relate to phylogenetic closeness among the species. The differences in sagitta morphology could be used as a tool for identification of fish species and could be useful for phylogenic study.

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Key words: Sagittal otolith, Morphology, Osteoglossiformes, Anguilliformes, scanning electron microscope

Study on diversity of phytoplankton and zooplankton in Trang River at Na Kluea Port, Kantang District, Trang Province

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The phytoplankton and zooplankton diversity were monitored between June 2013 to June 2014 in Trang River at Na Kluea Port, Kantang District, Trang Province from three stations; 250 meters toward the upstream from the port was St.1, front of the port was St.2 and 250 meters from the port toward the river mouth was St.3. Species of phytoplankton were found to

Division Bacillariophyta) Diatom (as 19 genera (*Asterionellopsis* sp., *Bacteriastrum* sp., *Chaetoceros* sp., *Corethron* sp., *Coscinodiscus* sp., *Ditylum* sp., *Guinardia* sp., *Gyrosigma* sp., *Helicotheca* sp., *Hemiaulus* sp., *Melosira* sp., *Mongeotia* sp., *Nitzschia* sp., *Odontella* sp., *Pediastrum* sp., *Planktoniella* sp., *Pleurosigma* sp., *Rhizosolenia* sp., and *Thalassionema* sp.) and Division Pyrrophyta) Dinoflagellate (as 5 genera)*Ceratium* sp., *Dinophysis* sp., *Gonyaulax* sp., *Protoperidinium* sp. and *Noctiluca* sp.(. Zooplankton were recorded to 3 Phylum as Protozoa (*Tintinnopsis* sp.), Arthropoda (Nauplius and Copepod) and Chordata (Fish Larvae). Moreover, species diversity (Shannon-Weaver Index) of phytoplankton were reported as 2.24 at St.2, 2.00 at St.3 and 1.92 at St.1, and zooplankton were showed as 1.01 at St.2, 1.10 at St.1 and 1.28 at St.3.

Key words: Diversity, Phytoplankton, Zooplankton, Trang River, Na Kluea Port

The preparation of titanium dioxide powder by the solvothermal method for photocatalytic degradation of methomyl

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Titanium dioxide (TiO_2) powder was prepared by the solvothermal method for photocatalytic degradation of methomyl under UV light irradiation was studied. Titanium isopropoxide ($C_{12}H_{28}O_4Ti$), ammonium hydroxide (NH_4OH) and nitric acid (HNO_3) were used as the starting materials. The mixed solution was diluted to 0.25M with ethanol (C_2H_5OH) and heated at 100 ^oC for 3h in a Teflon–lined stainless steel autoclave vessel.A laboratory set-up was designed to evaluate the optimal degradation process. The degradation performances is strongly dependent on the initial concentrations of methomyl, TiO_2 concentration and irradiation time. The effect of these parameters has been studied. The concentration of methomyl was measured using UV-VIS spectrometry (UV-VIS). The optimum conditions were obtained at 20 mg/L for initial methomyl concentration, 0.2 g/L for concentration of TiO_2 and irradiation time was 60 min.

Key words: TiO_2, solvothermal method, methomyl, photocatalytic, degradation

Fungal disease and endophytic fungion *Allium ascalonicum* Linn. in Amphur Laplae, Uttaradit Province

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Survey of *Allium ascalonicum* Linn. diseases caused by plant pathogenic fungi and endophytic fungi were done on September to December 2013. The objectives were to 1) investigate plant pathogenic and endophytic fungi diversity, 2) estimate plant disease epidemics under field condition. The pathogen was isolated from infected plant tissue by tissue transplant method and was cultured in Potato Dextrose Agar (PDA), whereas half-strength potato dextrose agar (1/2 PDA) was used for endophytic fungi isolation. Three isolations of pathogenic fungi were isolated, and were caused disease symptoms on healthy plant which diseases were occurred within 2 weeks. They were purple leaf spot, anthracnose and root rot diseasecaused by *Alternaria* sp., *Colletotrichum* sp. and *Fusarium* sp. which percentage of epidemics were 1.86, 6.75 and 3.87 %. Disease virulence of anthracnose was 1 – 3 degree or 12 - 52.5 percentage of leaf damage. Fifty-five isolates of endophytic fungi were isolated from the treatment of 1 % Clorox for 1 minute culture which presented the optimal condition which 47.27 % of endophytic fungi isolated from roots, 34.54 % from leaves and 18.18 % from stem. All of isolates was identified to 11 genera; *Arthrinium* sp., *Chaetomium* sp., *Colletotrichum* sp., *Corynespora* sp., *Curvularia* sp,.*Eupenicillium* sp., *Exerohilum* sp., *Fusarium* ssp., *Glomus* sp, Mycelia Sterilia and *Xylaria* sp. The future studies, all isolates of endophytic fungi will be studied on biological control efficiency with shallot fungal disease and DNA sequences of all endophyte will be analyzed.

Key words: *Allium ascalonicum*, Shallot disease, Epidemics, Endophytic fungi, Diversity, Isolate prevalence

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Enhancement of sensor response of acetone vapors based on platinum-loaded titanium dioxide nanoparticles

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Nanocrystalline powder property of unloaded titanium dioxide (TiO_2) and platinum-loaded titanium dioxide (Pt/TiO_2)and spin-coated nanocrystalline powder thin films have been prepared by flame spray pyrolysis. Structural and gas-sensing characteristics were performed by using scanning electron microscopy (SEM), X-ray diffraction (XRD) and High resolution transmission electron microscopy (HRTEM). Thin filmsis used extensively asthe acetone sensing material due to its change in electrical conductivity under analyst gas exposure.The gas sensing was studied at the operating temperatures of 300, 350 and 400 °C in dry air, respectively.It was found that Pt/TiO_2 sensing films showed higher response towards acetonegas than the unloaded TiO_2film.The response increased and the response time decreased with increasing of ethanol concentrations.

Key words: Flame spray pyrolysis, Thin films, Titanium dioxide, Acetone, Platinum

Litter dynamics and soil properties under upland para rubber (*Hevea brasiliensis* Mull. Arg) plantation, Lower Northern, Thailand

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This paper studies biomass, litter dynamics and soil properties under upland para rubber (*Heavea brasiliensis***)** plantation in lower northern, Thailand. Four permanent plots (50 m x 50 m) were established in 6 years para rubber plantation and sub-gridded into 100 quadrates (10 m x 10 m) for study. The monthly patterns of litterfall production were similar among four sites, with a peak during the dry period and dips during the wet period. The residual mass (% of the initial mass) of leaf litter had lost about 50% of their initial litter mass over the wet period. The average annual litter decomposition constant (*k*) was 0.10 ± 0.01 yr^{-1}. Litter fall production is a major process by which carbon and nitrogen are transferred from vegetation to the soil and which change soil properties in area. The comparison between upper soil (0-5 cm) and lower soil (0-20 cm) with % nitrogen, pH and % sand between upper soil (0-5 cm) and lower soil (0-20 cm) were not significantly different *p* value ($p>0.05$) and % carbon, % organic matter, % silt, % clay and soil saturated soil hydraulic conductivity (K*sat*) were significantly different *p* value ($p<0.05$).

Keywords: Litter dynamics, para rubber, biomass, soil properties

The efficiency of aquatic plants used in *raceway* pond in waste water treatment

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The objectives of this study were1) to determine the efficiency of three aquatic plants - Water hyacinth (*Eichorniacrassipes (Mart.) Solms*), Water lettuce (*Pistiastratiotes*L.), and Azolla (*Azollapinnata*R.Br.)-forwastewater treatment *in the raceway* pond (2 m x 0.25 m x 0.25m)and 2) to compare the flow rates into the *raceway* pondsystem. The results revealed that Water hyacinthshowedhighest efficiency in wastewater treatment comparing with the other. The

treatment efficiencies of Water hyacinthbased on Chemical oxygen demand (COD), Biochemical oxygen demand (BOD), and Total kjeldhal *nitrogen (TKN)*were71 %, 52 %, and 65 %, respectively. However, the treatment efficiencies for COD, BOD, and TKN on Water lettuce were 40 %, 52 %, and 42 %, respectively whereas Azollawere48 %, 59 %, and 34.2. Then, the Water hyacinthwas applied into the system to compare the efficiency of wastewater treatment among three wastewater flow rates; low (2 L/hr), medium (4 L/hr), and high (8 L/hr). The low flow rate presented the highest treatment efficiencies for COD (52 %), BOD (53 %), and TKN (51 %). This result was standardized when compared to the wastewater standards for some building type and building size.

Keywords : Wastewater treatment, *Raceway* pond, Aquatic Plants

The optimal condition of rice straw for biogas production

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This research aims to study the optimal condition and the efficiency ofbiogas production by chemical pretreatment and high temperature. Pretreatment condition was optimized by soaking the rice straw at 3%, 6% and 9% by wt (NaOH), boiling at 60, 80 and 100ºC with operation time for 30, 60 and 90 minutes, respectively. The results showed that the optimal pretreatment of rice straw was soaking at concentration of 9%NaOH, boiling at 100ºC, 90 minutes for reducing sugar yield 0.26 mg/ml In addition, the efficiency of the maximal biogas production was producedat 356.11ml/day .The best pretreatment of rice straw for the enhancement of biogas yield.

Keywords: Physical-Chemical Pretreatment, rice straw, biogas production

Occurrence of anthracnose disease on English Ivy caused by *Colletotrichum* with curved conidia in Thailand

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Anthracnose disease occurred on ornamental English ivy in Chiang Mai and Chiang Rai provinces in Northern Thailand. Symptoms developed near the margins of the leaves or began as circular necrotic spots that expanded into circular or irregular necrotic areas. Acervuli in which single-cell, curved, hyaline conidia and dark brown setae were produced appeared as black specks scattered on the necrotic surfaces. Two isolates from Chiang Mai and one isolate from Chiang Rai were obtained from the leaf lesions by using the single-spore isolation technique, and were identified as *Colletotrichum trichellum* based on morphological and cultural characteristics. The ITS sequence of an isolate was analyzed to confirm the species identification. Multigene phylogenetic analysis of all isolates will be published elsewhere. This is the first occurrence of *C. trichellum* causing anthracnose of English ivy in Thailand.

Keywords: Anthracnose; *Colletotrichum trichellum*; English ivy; *Hedera helix*

First report of *Endophyllum* causing *Emilia* rust in Thailand

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A rust fungus *Endophyllum* (Pucciniaceae) was found on *Emilia sonchifolia* (Asteraceae). The disease is characterized by large pale yellow spots with masses of bright yellow-orange, powdery spores, erupting from hypophyllous sori. Spermogonia were absent. Aecia are telioid, amphigenous, subepidermal, without paraphyses or peridia, cupulate, and peridiate. Aeciospores develop in chains, are teloid, globose to polygonal, orange-yellow fresh, verrucose, and germ pores are indistinct. This is the first report of a rust disease on leaves of *Emilia* in Thailand.

Keywords: *Emilia*, *Endophyllum*, Pucciniaceae, rust disease

A new *Maravalia* rust in Thailand

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The rust genus *Maravalia* Arthur (Chaconiaceae, Urediniomycota) is characterized by pale, single-celled, thin-walled teliospores that elongate to form external basidia. It is one of the most ancient rust genera. *Maravalia* infects hosts in several plant families, with 16 species occurring on hosts in the Fabaceae. A new species of *Maravalia* rust on *Millettia extensa*, from Thailand is proposed in this study. Uredinia are surrounded by peripheral dense paraphyses;urediniospores are asymetrically obovoid to ellipsoid, with a concave side. Telia are similar to and replacing uredinia; teliospores are short-pedicellate, one-celled, obpyriform, ovoid to ellipsoidal,and germinate apically into two-celled basidia. Pycnia and aecia are unknown. The proposed new species is distinct from other species by producing two-celled basidia while others produce four-celled basidia. This is also the first report of *Maravalia* to be described on *Millettia*, in Thailand.

Keywords: Fabaceae, *Maravalia*, *Millettia extensa*, rust disease

Tar spot disease of *Dalbergia* in Northern Thailand

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East Indian Rosewood (*Dalbergia lanceolaria*; Fabaceae) is a common tree in Northern Thailand forests. In 2014, during the annual survey of fungal diseases, a plant was observed exhibiting typical tar-spot symptoms and bearing amphigenous black stromata on the upper surface of host leaves. Light microscopy revealed stromatal perithecia containing paraphyses and asci with eight hyaline, unicellular ascospores. A fungus detected in symptomatic tissue was identified as *Phyllachora* (Phyllachoraceae). *Phyllachora* infects hosts in various plant families, with eight species occurring on *Dalbergia.* This is the first report and completed description of this species of *Phyllachora* on *Dalbergia* in Thailand.

Key words: *Phyllachora*, *Dalbergia*, tar spot disease

Aristastoma leaf spot on desert rose in Thailand

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A species of *Aristastoma* was isolated from foliar lesions of Desert Rose (*Adenium obesum*; Apocynaceae) that was found in a commercial nursery in Chiang Mai, Thailand, where 80% of the crop was affected. Plant foliage had irregular, oval to circular, rusty brown, amphigenous, necrotic lesions 5 to 15 mm in diameter. Large leaf spots developed tan centers. The fungus has pycnidial conidiomata, epiphyllous, separate, globose, dark brown, semi-immersed, unilocular, thin-walled with textura angularis. Ostioles are central and circular. Conidia are hyaline, cylindrical, taper at the apex, and have truncate bases, and 0-1 transverse eusepta. This is the first report of *Aristastoma* on *Adenium obesum* from Thailand. The causal fungus was identified as a new species namely *Aristastoma adeniae*.

Key words: *Aristastoma*, *Adenium obesum* and Desert Rose

First report of tomato gray leaf spot disease caused by Stemphylium solani in Northern Thailand

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Gray leaf spot is one of the most important disease of tomatoes. It is caused by Stemphylium solani and can attack leaves, petioles, and stems.Yield is reduced, and in some cases entire crops have been destroyed over a short time. Because of its prevalence, distribution, and severity, the disease has become the major factor limiting tomato production in this region. Disease symptoms were commonly observed on seedlings and plants. On leaves the disease first appears as circular to elongate dark specks. As the spots enlarge, they become gray and bright. Old lesions dry and usually crack. Severely infected leaves turn yellow and then die and drop. Lesions on petioles and stems are elongate. Disease severity is generally higher following the beginning of fruiting.This is the first report of *S. solani* causing gray leaf spot on tomato grown in Northern Thailand.

Key words: Gray leaf spot, Stemphylium solani

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In vitro effects of *Streptomyces*-culture media against various fungal pathogens causing postharvest diseases of maize and fruits

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The antagonistic activities of six strains of *Streptomyces* species, isolated from natural soil samples from Suthep-Pui National Park, Chiang Mai, Thailand, and named NSP 1-6, were evaluated *in vitro* against various fungal pathogens causing postharvest diseases of maize and fruits. *Streptomyces* species inhibited pathogen growth from 45.7 – 72.8% for maize and 35.7 – 82.4% for fruits when screened using the dual culture method. In addition, the ability of *Streptomyces* culture media to inhibit the conidial germination of two types of pathogens from each host: *Acremonium* sp. and *Fusarium* sp. from maize, and *Alternaria* sp. and *Pestalotiopsis* sp. from fruits, was tested. Conidial germination was checked at 3, 6, 9, 12 and 24 h after treatment. The antifungal activities of chitinase produced by *Streptomyces* species were determined using enzyme production medium (EPM). Cultures were incubated by shaking at 35 ºC for 5 d, and divided into two parts; non-filtered culture medium (NF) and filtered culture medium (F). Among the six *Streptomyces* species tested, both culture media of isolates NSP2 and NSP5 inhibited conidial germination of all pathogen isolates; the media completely inhibited *Fusarium* sp. and *Pestalotiopsis* sp. during the test period. In addition, the culture media produced a 70% inhibition of *Acremonium* sp. and *Alternaria* sp. that gradually decreased to 50% from 6-24 h. The *Streptomyces*-culture media did not inhibit germ tube elongation but caused morphological abnormalities.

Keywords: *Streptomyces*, postharvest disease, culture medium, antifungal activity

Cercospora species on Euphorbiaceae from Northern Thailand

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The genus *Cercospora* is one of the largest genera of hyphomycetes, which has well-known associations with leaf diseases on a wide range of host plants. The fungi treated in this study were collected and isolated from Euphorbiaceae leaves in Northern part of Thailand *viz.*, Chiang Mai and Chiang Rai. Species were initially identified based on morphological and cultural characteristics. However, identifications were confirmed by generating partial sequences of the internal transcribed spacer and calmodulin regions. Phylogenetic tree analyses were perfomed in MrBayes showing that all of these isolates could be distinguished from other taxa. By integrating the morphological and molecular data sets, *Cercospora geniculatum* was proposed as a new species in this report.

Key words: *Cercospora geniculatum*, Cercosporiod, Euphorbiaceae, taxonomy.

Diversity of cercosporoid fungi on Poaceae in Northern Thailand

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An investigation of the diversity of cercosporoid fungi was carried out by isolating fungi from Poaceae leaves in Chiang Mai, Chiang Rai and Lamphum provinces during September, 2013–August, 2014. Each fungus was directly isolated using the single spore isolation technique. A total of 21 cercosporoid fungal isolates were found on cogon grass (*Imperata cylindrical*), crowfoot grass (*Dactyloctenium aegyptium*), para grass (*Brachiaria mutica*) and mountain grass (*Hymenachne pseudointerrupta)*; seven differentiated species were determined morphologically. Pure cultures are being maintained in a culture collection at the Entomology and Plant Pathology Department, Faculty of Agriculture, Chiang Mai University, for further study of phylogenetic relationships.

Keywords: cercosporoid, *Imperata cylindrical, Dactyloctenium aegyptium, Brachiaria mutica, Hymenachne pseudointerrupta*

Phytophthora blight and fruit rot of passion fruit in Thailand

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During the period 2013–2014, a survey of Passion fruit diseases was conduct at Royal Project stations such as, Khunwang, Hungsompoy, Maethanuier, Pangdangnai, Maetho, Mokcham and Sango. Phytophthora Blight and Fruit Rot of Passion fruit was found in all of these areas. The fungus that causes this disease is a soil inhabitant and produces spores on the soil surface in warm wet weather. These spores are then splashed on to the lower parts of the vine where the infection begins. Wind-driven rain spreads the spores further up the vine. Infected mature leaves show large patches which are translucent at first and then turn light brown. Shoots may die from the tip and appear black. Fruit infection is characterized by large grey-green water soaked spots which enlarge to cover much of the surface. Infected leaves and fruit usually fall from the vine. As the disease progresses, it can lead to irreversible wilting and loss of foliage. Isolation of the causal agent of the disease was conducted and a *Phytophthora* species was isolated from the leaves and fruits and was identified as *Phytophthora nicotianae*. This is the first report of Phytophthora blight and fruit rot of Passion fruit in Thailand.

Key words: Phytophthora blight, fruit rot, *Phytophthora nicotianae*, Passion fruit.

First report of powdery mildew on *Bauhinia purpurea* (Leguminosae) caused by *Pseudoidium bauhiniae* in Thailand

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During a survey of powdery mildew fungi in Northern Thailand, the typical signs of powdery mildew - dense, white mycelium in irregular patterns often covering almost the entire upper surface of the leaves and pod - were observed on *Bauhinia purpurea* (Leguminosae). Microscopic observations revealed mycelium with lobed appressoria and large, approximately cylindrical conidia that measured 30 to 42.5 x 12 to17.5 µm. The short germ tubes were at one

end of the conidium and terminated in a lobed appressorium. Conidiophores were straight, ca. 32.5 to 57.5 µm in length, with cylindrical foot-cells. Based on these characteristics the fungus was identified as *Pseudoidium bauhiniae* (Syn. *Oidium bauhiniae*). This is the first report of powdery mildew on *Bauhinia purpurea* in Thailand.

Key words: Powdery mildew, *Pseudoidium bauhiniae*, *Bauhinia purpurea*, Leguminosae, taxonomy.

First report of *Gonatophragmium* ring spot on mulberry in Thailand

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A species of *Gonatophragmium* was isolated from foliar lesions of Mulberry (*Morus alba*; Moraceae) in many orchards in Chiang Mai, Thailand in 2014. The fungus causes large zonate leaf spots. Colonies are effuse, grey to olivaceous, hypophyllous. Conidiophores are mostly nomonematouse, branched, flexous, with nodose swellings which often proloferate as short lateral branchlets. Conidiogenous cells are polyblastic sympodial, integrate, terminal and intercalary, and denticulate. Conidia are solitary, dry, acropleurogenous, cylindrical to clavate, slightly curved, and pale brown with three transverse septa. The causal fungus was identified as *Gonatophragmium mori*. This is the first report of *Gonatophragmium* on Mulberry in Thailand.

Key words: *Gonatophragmium mori*, zonate leaf spot, *Morus alba*, Mulberry

Optimization of large-scale culture conditions for the production of *Cordyceps militaris*

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Cordyceps militaris, commonly known as the orange caterpillar fungus, is being investigated with great interest. Cultivation of this fungus has been practiced on a large scale in order to fulfill a demand for the commercial use. The process involves first cultivating *C. militaris* in a liquid medium as the inoculum, followed by the subsequent cultivation of *C. militaris* in solid-state cultures using barley seed + PDB + Egg + *Bombyx mori* larva as the substrate. The factors affecting mycelial growth and the fruiting body production were analyzed. The optimal temperature and light regime for mycelial growth and the fruiting body production were 22-25 °C in dark for 4 weeks, followed by 15-18 °C in light (500 lux) for 2 weeks and finally 22-25 °C in light (600-1,000 lux) for 6 weeks. This is a very effective procedure for cultivation of *C. militaris.*

Key words: medicinal mushroom, *Cordyceps militaris*, large-scale culture

Screening and optimization of protease production by proteolytic bacteria for deproteinization of crab shell for green chitin production

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The production of chitin by using enzyme-producing microorganisms is a green technology in the utilization of shellfish processing wastes. In this study, protease-producing microorganisms

were isolated from soil samples in four areas of Thailand using a medium containing 2% crab shell powder, 0.1% K_2HPO_4, 0.05% $MgSO_4.7H_2O$ and 2% agar. Forty-five isolates were obtained from the first screening and twenty-nine strains formed clear zones on the medium. These isolates were used to study protease production in a liquid medium containing crab shell powder, and the deproteinization of crab shell wastes. It was found that after being shaken at 37 °C for 2 d, two isolates each from Suratthani Chiang Mai had high protease activity. The ECM04 isolate had the highest protease activity (2.64 unit/ml). This isolate was used to study the optimization of conditions for deproteinization for chitin production. The optimal procedure for protease production included: shaking at 37°C for 36 h in 100 ml of a liquid medium containing 7% crab shell powder, 0.1% K_2HPO_4, 0.05% $MgSO_4.7H_2O$ and 3% carboxymethyl cellulose (CMC), at pH 8.0. In terms of protein removal from crab shell wastes, it was found that isolate ECM04 can deproteinize 63.78% of the protein after 3 d, and this coincided with maximal protease activity (3.74 unit/ml).

Keywords: protease production, proteolytic bacteria, green chitin, deproteinization

Growth evaluation test in different condition of *Cordyceps sp.*

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Cordyceps sp. is an entomogenous fungus belonging in to Ascomycotina ; Hypocreales : Clavicipitaceae. This fungus is a popular souse of medicine in many asian country. This fungus produce a compound known as corgycepin. It is belive that this fundus can possessed many important pharmacological activities. It can modulate immune responses , inhibit the growth of tumor cells, enhance hepatic energy , promote the secretion of adrenal hormones and possess hypotensive and vasorelaxant activities this study aimed to study some physiological requirements for the growth and cultivation of *Cordyceps* sp. 4 media namely PDB with peptone , PDB with yeast extract , milk insect glucose homogenous broth (MGI) , insect glucose homogenous broth (GI) were use to determine the effect of difference media on the mycelia growth of difference isolate of *cordyceps* sp. solid media were use for fruiting production. Result review that. GI media was the best for isolate 2 PDB with the yeast extract is the best for isolate 4 and 5. isolate 3 has the same reaction with every type of media but do not grown in GI media. In solid media primodia develop in all the media but none developed into fruiting body.

Key words: *Cordyceps* , corgycepin , entomogenous fungus , Ascomycotina

Study on the growth of *Pleurotus giganteus*

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Pleurotus giganteus is an edible mushroom in nature but it is not enough for demand. Studies on the growth the mushroom are needed. The objective was to study the physiological requirement and cultivation of *Pleurotus giganteus* on PDA and PDB with difference pH levels (3 , 5 , 7 and 9) and different formulation of media such as Potato dextrose broth (PDB) , Corn flour media , Corn flour media with dextrose , Coconut media , Coconut with dextrose , Rice bran and rice bran with dextrose. To determine the effect on fruiting body production, two experiments were done. In the first experiment, the following casing materials were used : ordinary loam soil, Soil with bamboo leaf , Soil with rain tree leaf and ordinary loam soil with 10 % amino were used. In the second experiment ordinary soil was used but added with additional nutrients such as: amino with *Rhodopseudomonas* (0.5 % and 1 %) and nano KS1 (0.5 and 1 %). Results showed that PDA at pH 7 was the highest colony among treatment and PDB at pH 7. The use of ordinary soil with 10 % amino can produce the highest number of fresh and dry weigh and the quantity of the fruiting bodies. Nano - KS1 at 1 % could promote the highest quantity and weight of fruiting bodies.

Key words: giant mushroom, media, fruiting bodies

Big 6 vegetable diseases in Champasak, Laos

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Vegetable production is a key agricultural industry both for smallholders and business owners in Lao PDR, with a growing and profitable regional export market. Despite this, there has been virtually no extension support for plant disease diagnostics and management. Over the past six years of intensive production there has been a gradual increase in the incidence and severity of diseases, leading to a decline in productivity. Since 2009, The Crawford Fund of Australia has assisted Australian and British volunteers with capacity building efforts in plant disease diagnostics and advice on appropriate Integrated Disease Management (IDM) strategies in Lao PDR, with efforts extended to the province of Champasak since 2012. In Champasak alone we

estimate there to be more than 100 vegetable diseases, on more than 50 crops. The most important of these have been identified as: Rhizoctonia collar and root rot (*Rhizoctonia solani*), Bacterial Wilt (*Ralstonia solanacearum*), Root Knot Nematode (*Meloidogyne* spp.), Phytophthora root rot of chilli (*Phytophthora capsici*), Late blight of tomato (*Phytophthora infestans*) and the most recently detected Sclerotinia head rot of cabbage (*Sclerotinia sclerotiorum*). We would estimate these six diseases, which have very wide and important host ranges, cause more than 70% of recorded yield losses amongst vegetables on the plateau, with the recent detection of Sclerotinia head rot highlighting that quarantine may not be entirely effective to date (it had never previously been recorded in the province, since surveys began in 2009).

Key words: Plant pathology, crop disease diagnostics, food security

Use of fungicides and Actinomycetes to control Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*

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Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), is one of the most important diseases of tomato. The objective of this study was to control Fusarium wilt by using actinomycetes and fungicides *in vitro*. Eight fungicides were divided into two groups: group one consisted of contact fungicides such as iprodione, mancozeb, propineb and quintozene+etridiazole; and group two comprised of systemic fungicides such as prochloraz, carbendazim, myclobutanil and propiconazole+difenoconazole. Using the poisoned food technique at the recommended concentrations, carbendazim) 250 ppm(and prochloraz)500 ppm(produced 100% inhibition of *Fol* mycelial growth and spore production. In addition, mancozeb)3,200 ppm(and propineb)1,050 ppm(inhibited conidial germination by 100% at 3, 6, 9, 12 and 24 h. Six actinomycete isolates, NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6, were studied. According to the dual culture method, all six isolates were effective in inhibiting the mycelial growth of *Fol*, with fungistatic activities ranging from 63.14 - 72.57%.

Key words: *Fusarium oxysporum* f.sp. *lycopersici*, fungicides, actinomycetes

Diversity of endophytic fungi associated with palm trees

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Endophytic fungi - the fungus that live within plant tissues and without causing any symptoms - were isolated from 12 species of palm trees in King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. And , 65 isolates were obtained from leaves, petioles and roots of palm trees, including 45 identified endophytic fungi species as: cladosporium spp., phialophora spp., pestalotiopsis spp., phoma spp., Phomopsis spp., Nigrospora spp., Xylaria spp., Fusarium spp., Rhizoctonia spp. and Colletotrichum spp. and 20 isolates belonging to mycelia sterilia fungus.

Key words: palm trees, endophytic fungi

Screening and characterization of phytase-producing thermotolerant bacteria from Thai soils

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Phytase catalyses the sequential release of phosphate from phytic acid (phytate), the organic stored form of phosphorus present in various seeds and grains that commonly used as raw materials in foods and feeds. Monogastric animals are unable to degrade phytate in crops. Therefore, to enhance phytate degradation by phytase enzyme in crops is useful to improve the absorption of nutrients. Furthermore, the most of industrial processes are carried out at high temperature that thermostable enzymes would give an advantage. This research was aimed to isolate, screen and characterize the phytase-producing thermotolerant bacteria. Eighty strains of phytase-producing thermotolerant bacteria were isolated from 56 soil samples collected from 30 provinces, Thailand. All isolates were Gram-positive, spore forming rod-shaped bacteria and

belonged to *Bacillus*. They exhibited the ability to degrade sodium phytate based on the counterstaining technique. The phytase production of isolates in shake flask fermentation was carried out and the enzyme activity were screened at 45 °C. The phytase hydrolysis capacity of the isolates were 1.0-3.7 and phytase activity ranged from 0.08 to 24.42 units/ml. The selected isolates were characterized taxonomically based on the phenotypic characteristics and 16S rRNA gene analysis.

Key words : Phytic acid, phytate, phytase, thermotolerant bacteria

Screening of antagonistic yeasts against fungal plant pathogens causing disease in vegetables

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A total of 145 epiphytic yeasts isolated from various fruit surfaces were selected for antagonistic screening against fungal pathogens such as *Alternaria brassicicola*, *Fusarium oxysporum* f.sp. *lycopersici*, *Pythium* sp., *Rhizoctonia solani* and *Sclerotium rofsii*. In vitro screening using the dual culture technique was undertaken to assess the antagonistic potential of the yeasts. Isolate CMY013 showed 63.8 % growth inhibition against *A. brassicicola*, isolate CMY044 showed 74.1 % against *F. oxysporum* f. sp. *lycopersici*, isolate CMY064 showed 84.4 % against *Pythium* sp., isolate CMY048 showed 71.8 % against *R. solani* and isolate CMY006 showed 68.8 % against *S. rofsii.* This study identified those five yeast isolates as promising biological control agents for further testing against diseases in vegetables.

Keywords: Antagonistic yeast, epiphytic yeast, biocontrol, fruits surface

Cytotocixity and antimicrobial properties of nanocomposites of *Chaetomium globosum* extracts: Preliminary studies

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The use of nanotechnology in production of bioproducts for agriculture is fast developing because of its believed improved efficiency and delivery mode. One of the more potent biocontrol agents is *Chaetomium globosum* due to its broad antimicrobial properties and high efficiency in controlling plant pathogens as well as enhancing plant physiological activities. Nanocomposites from different extracts of C. globosum have been produced through electrospinning and are being tested for their use in biocontrol. These preliminary studies aimed to find out the cytotoxicity of nanocomposites of ethyl, methyl and hexane extracts of *C. globusom* using the brine shrimp assay as well as do some antimicrobial properties against some bacteria and fungi using disk and slide assay methods. Results of the preliminary studies revealed that the nanocomposite with methanol extract of *C. globosum* had higher LC50 on the 24th hour compared to nanoethanol and nanohexane extracts. The nanocomposites with different extracts did not inhibit the growth of *Escherichia coli* and *Pseudomonas aerouginosa*. The hyphal growth from sclerotia of *Sclerotium sp* was inhibited by the nanocomposites of the different extracts of *C. globosum*.

Key words: nanotechnology, Chaetomium globosum, brine shrimp assay, nanocomposites

Basis for development of biotechnology for plant protection means in Georgia

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Pest organisms cause the great damage to agriculture in Georgia. It becomes necessary to carry out the control measures against them. The plant protection from pest organisms requires the development of integrated pest management (IPM) system, where the biological agents will take the important place for human and environment. The greenhouse whitefly (GWF), *Trialeurodes vaporariorum* polyphagous insect is the most significant pest of agricultural crops in open and closed holdings. The specialized parasitoid of GWF – *Encarsia*, *Encarsia formosa* develops inside the body of whitefly larva. The local population of parasitoid has revealed (Tbilisi, Botanical gardens) and has used in experiments. The *Encarsia* biological effectiveness (BE) on vegetable crops (tomato, cucumber) and ornamental plantings at winter-spring period is 85-90% and 52-60% to the fall period. The results of study on the joint action of_entomopathogenic nematode (EPN) – *Steinernema feltiae* (introduced from Israel) and parasitoid *E. formosa* to the GWF have been established. The mass production of *Encarsia* in laboratory has elaborated. Pest insects – *Coccids* are widely distributed in Georgia. They damage the agricultural crops and ornamental plants. The relationship of EPN, *S. feltiae* to *Coccids* has studied and there is the prospect *S.feltia* using to control the *Coccids* in closed and open ground farms. *S.feltia* was tested also to main pest of vine the grape berry moth, *Lobesia botrana.* There is the preliminary data on biotechnology of nematode formulation on the base of local EPN, *S.feltia* "Georgian strain", tentatively called *"Geo-nema*". The accumulation of biomass by growing of EPN *in vivo* on laboratory cultures of *Galleria mellanolla* and *Tenebrio molitor* has elaborated. Bioformulation will be economically accessible to local manufacturers of environmentally friendly product. At present the fall webworm (FWW), *Hyphantria cunea* is a very dangerous quarantine pest insect, widely spread in urban holdings, where the use of chemical pesticides are prohibited. The formulations on base of entomopathogenic organisms (fungi, bacteria, viruses, EPNs) have examined. The possibility of using sex pheromones (Russia) for complex control to FWW at first time for Georgian conditions has established. The possibility of using entomopathogenic fungi to the very important pest insect for vegetable and technical cultures - the Colorado potato beetle, *Leptinotarsa decemlineata* has elaborated. The action of strain, *Beauveria bassiana* LRC_{107} (introduced from Canada), enriched KNO_3 (Joint project, Durmishidze Institute of Biochemistry and Biotechnology) to the different instars pest larvae and imagoes in natural conditions has studied. The average BE was achieved to 70-80%. The sunflower broomrape, *Orobanche cumana*, root-parasitic weed plant is the major pest of sunflower crops in Georgia. At present the investigations are carrying out on search the local pathogenic fungi at the *O. Cumana* populations. The high virulence isolate from genus *Fusarium* strain will serve as the base for mycopesticides production. The above mentioned biological agents are considered as basis for development the formulations, which offer a desired integrated pest management (IPM) - compatible alternative to broad-spectrum unselective chemical insecticides. The non-toxic and environmentally safe means gives possibility obtaining the ecologically pure production, which is a very important social problem.

Key words : biotechnology, pest managemant